

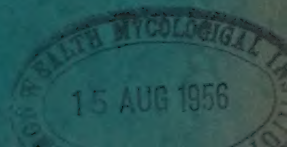
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MELBOURNE

GRAZING MANAGEMENT OF NATURAL PASTURES AT TRANGIE, NEW SOUTH WALES

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(Manuscript received December 12, 1955)

Summary

Results of a 5-year grazing experiment on *Stipa-Chloris* natural pasture are presented. Treatments were three rates of stocking each tested with continuous grazing, autumn deferment, and spring deferment. These were studied in relation to: (a) body weight and wool production of Merino wethers; (b) available feed; (c) density and basal area of the perennial grasses.

Lighter stocking (one sheep/2 ac) showed little superiority in production per animal under the generally favourable rainfall conditions. There was only one interval of acute feed shortage in which heavier stocking (one sheep/ac) caused extreme body-weight loss and lower fleece weight. The spelling treatments gave no advantage over continuous grazing, either to sheep or to pasture.

Effect of stocking rate on perennial grasses mainly depended upon the amount of rainfall received during certain growth stages of the species, e.g. the winter vegetative growth of *Stipa*, and the March-April maturation of *Chloris* and *Digitaria*. Generally, lighter stocking was favourable to plant density and basal area in seasons of average or below average rainfall; heavier stocking was best in wet seasons. Probable reasons for these relationships are discussed.

I. INTRODUCTION

Trangie lies towards the north-western extremity of the main belt of red-brown earth soil complex extending southward through New South Wales to Victoria. The western edge of this belt is largely grazing country with an average annual rainfall ranging from 17 to 19 in., featuring erratic seasonal incidence. The wool and fat stock production of the area is a large contributor to the Australian industry.

Since the introduction of domestic grazing stock the perennial grasses of these pastures, chiefly *Stipa*, *Chloris*, and *Digitaria* spp., have undergone a steady decline. In view of this decline, the Commonwealth Scientific and Industrial Research Organization and the New South Wales Department of Agriculture jointly established a grazing experiment in March 1948 to study management problems of the pastures. The primary objective was to investigate methods of maintaining the pastures in a vigorous state while

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securing the highest practicable level of productivity in terms of sheep and wool.

The experiment was located at the Trangie Agricultural Experiment Station on typical light soils of the region. The soils have been described by Downes (1947). Biddiscombe, Cuthbertson, and Hutchings (1954) have described the pasture community, in which *Stipa falcata* Hughes, *Chloris acicularis* Lindl., and *Digitaria coenicola* (F. Muell.) Hughes are the main perennial grasses, and *Medicago* spp. and *Erodium cynorum* Nees the main winter annuals.

II. EXPERIMENTAL DESIGN

Treatments imposed were three rates of stocking each tested with continuous grazing, autumn deferment, and spring deferment. Rates of stocking were:

- (a) One sheep to 1 ac.
- (b) One sheep to 1.5 ac (estimated district average).
- (c) One sheep to 2 ac.

For convenience in presentation these rates will be designated as heavy, moderate, and light stocking respectively, using the terms in a relative sense.

Autumn and spring deferment involved removal of the sheep for 6 weeks following the first effective seasonal rain. The treatments aimed at assisting early development of winter or summer species either germinating or making regrowth at these stages.

With a unit flock size of six sheep, plot sizes for continuous grazing were 6, 9, and 12 ac, and for deferments 46/52 of these acreages to provide equivalent annual stocking rates. The experimental area of approximately 300 ac was laid out on the basis of line transect data so that treatments, over four replications, were similar in mean basal area of perennial grasses. Flooding on four occasions between April and November 1950 caused considerable plant mortality and loss of seed reserves on some of the plots. These plots were eliminated from the experiment by reducing replication to three.

The original grazing flock consisted of 2-year-old Merino wethers allocated to treatments at random from groups stratified with respect to body weight. Dry sheep were selected in preference to breeding ewes in an attempt to stabilize replacement, and factors of nutrition and body weight between flocks. A replacement flock was maintained at the moderate stocking rate on an area adjacent to the experimental site.

III. EXPERIMENTAL TECHNIQUES

(a) Sampling of Available Feed

Available feed is defined as the amount of feed offering to the sheep at the time of sampling. No attempt was made to measure separately

either pasture yield, grazing intake, or feed losses due to other factors. Such data would have doubtful accuracy because of: (i) variable rates of leaf fall and decomposition; (ii) loss of dry plant material by wind action; (iii) fluctuating rabbit numbers; (iv) sampling error on large plots.

Available feed included the following fractions as sampled: (1) green feed; (2) dry feed, or the sum of standing and fallen dry material (including *Medicago* burr). Samplings were made every 2 months to March 1952, and thereafter in July, September, January, and March. Quadrats (35.1 by 35.1 in.) were placed at random and equal numbers of samples taken from each plot. Sampling intensity varied from eight to 16 quadrats per plot, depending on seasonal changes in the amount of available feed. Green and dry feed fractions were collected separately from each quadrat. Species contribution to green feed was obtained by weight estimate method in the field.

From March 1952 the sampling technique was modified to follow the ranked set method (McIntyre 1952); botanical composition was then obtained by hand-separation.

Damage by rabbits was recorded on individual quadrats in the course of sampling.

(b) *Plant Density and Basal Area Measurement*

These measurements were taken in March each year and were applied only to the perennial grasses. On each plot the data were obtained from six permanent quadrats (2 by 1 m) initially located at random. Established plants were counted, and density expressed as number of plants per square metre. Average basal diameter of each plant was measured by pressing a rule through the crown as near as possible to ground level. Basal area, expressed as a percentage of total (quadrat) area, was calculated from the diameter measurements. Perennial grass seedlings were counted separately but not included in the density totals for final analysis.

All pasture data for the period May 1950 to January 1951 were discarded from analysis because of flood damage and amendment of the design (reduction from four replicates to three). However, the appropriate grazing schedule was continued during this period on the plots subsequently kept under experiment.

(c) *Animal Production Measurement*

Sheep were shorn prior to entry into the experiment in March 1948. To conform to normal shearing time for the district, the first wool data were obtained in August 1948 and represented approximately 6 months' growth. Thereafter, measurements were made on the full fleece of 12 months' growth. Following shearing in July 1949 and 1950, three sheep of each unit flock were replaced by 18-month-old wethers. Subsequently annual replacement was two sheep per unit flock.

Body weights were recorded every 2 months throughout, approximately at the time of pasture sampling in most instances. Data for the period May 1950 to January 1951 were not analysed.

IV. CLIMATE

General features of climate for the period 1948-1952 have been discussed by Biddiscombe, Cuthbertson, and Hutchings (1954). While their consideration of averages indicated the generally favourable rainfall of the period, these mask the important aspect of annual and seasonal variability.

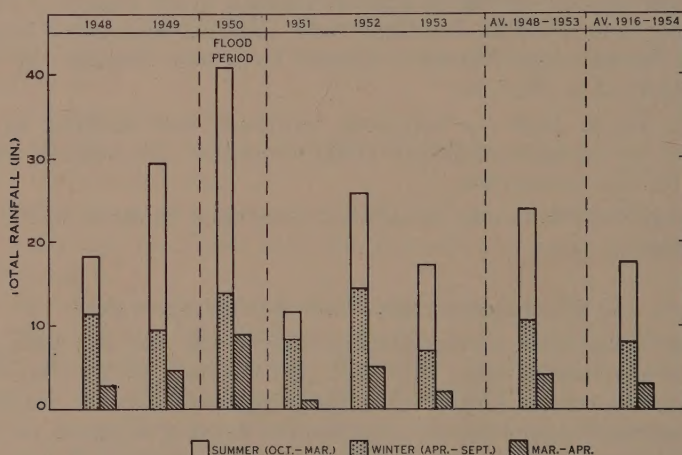


Fig. 1.—Seasonal rainfall at Trangie Agricultural Experiment Station during the experimental period. Annual rainfall in all cases (including the 1916-1954 average) is calculated on a March-February basis.

Disregarding the interval March 1950 to March 1951, in which flooding caused a break in the experiment, the dominant feature of the 1948-1953 period was the alternation of relatively wet and dry years (Fig. 1). Summer rainfall (October-March) and March-April rainfall proved the most variable, and also provided the higher frequency of dry conditions. Winter rainfall (April-September) remained above average except in the final year.

V. RESULTS

(a) *Effect of Stocking Rate on Available Feed and Animal Production*

(i) *Feed Levels.*—In general, green feed was greater under light stocking as compared with heavy stocking (Fig. 2). Significant differences ($P < 0.05$) in favour of light stocking were recorded for all samplings except those of March, May, and July 1948. The differences thus occurred

at all feed levels to the maximum average of 26.1 cwt/ac. Under moderate stocking green feed was intermediate, generally at a level significantly above heavy stocking and below light stocking.

Significantly greater dry feed was obtained from light stocking in 16 of the 28 samplings. This effect sometimes appeared in January, but occurred more frequently in March. In the drier winters of 1951 and 1953 the differences were maintained into July and September. Usually all three stocking rates differed significantly during these intervals. The data therefore indicated an appreciable consumption of dry feed when green material fell to a low level, and also when late summer pasture became tall and rank.

A high population of rabbits was present on the area during early summer of 1949-50, and during the winter of 1951 and the following summer. At these times the overall consumption by rabbits probably exceeded the consumption by sheep, but current estimates showed that rabbit grazing was uniformly spread over the treatment areas. It is considered that treatment comparisons for these periods remained valid because the main effect of rabbits was a reduction only in the general level of available feed.

(ii) *Body Weight and Wool Production.*—Marked treatment effect on body weight was evident at low levels of green feed (Fig. 2), when body weight could not be sustained on mainly dry feed. All flocks received a supplementary maintenance ration for part of the dry winter of 1951, but the first marked differences in body weight occurred at January 1952. At this stage there was a weight advantage from light stocking, all three rates being significant. Shortage of green feed was most pronounced in March, when sheep at heavy stocking showed an extreme loss in body weight and were at the point of requiring hand-feeding. Following a rapid recovery in all groups during the favourable autumn, a significantly higher body weight was maintained at light stocking for the remainder of the experiment.

The flock at moderate stocking remained lowest in body weight until partial replacement in 1949. After September 1951 the weight level for this treatment was intermediate, without showing significant advantage over heavy stocking.

Light stocking favoured a greater body weight increase between September and July. This treatment gave an average increase of 15.8 lb for the 3 years of complete record (to July 1949, 1952, and 1953). Body weight gain therefore exceeded fleece weight by about 3 lb for the period because the corresponding average fleece weight was 12.9 lb (Fig. 2). The average gains for moderate and heavy stocking were 12.6 and 12.1 lb respectively; here wool growth accounted for the whole of the increase.

Significant differences in greasy fleece weight were recorded only at the 1952 shearing (Fig. 2), when the heavier fleeces came from light

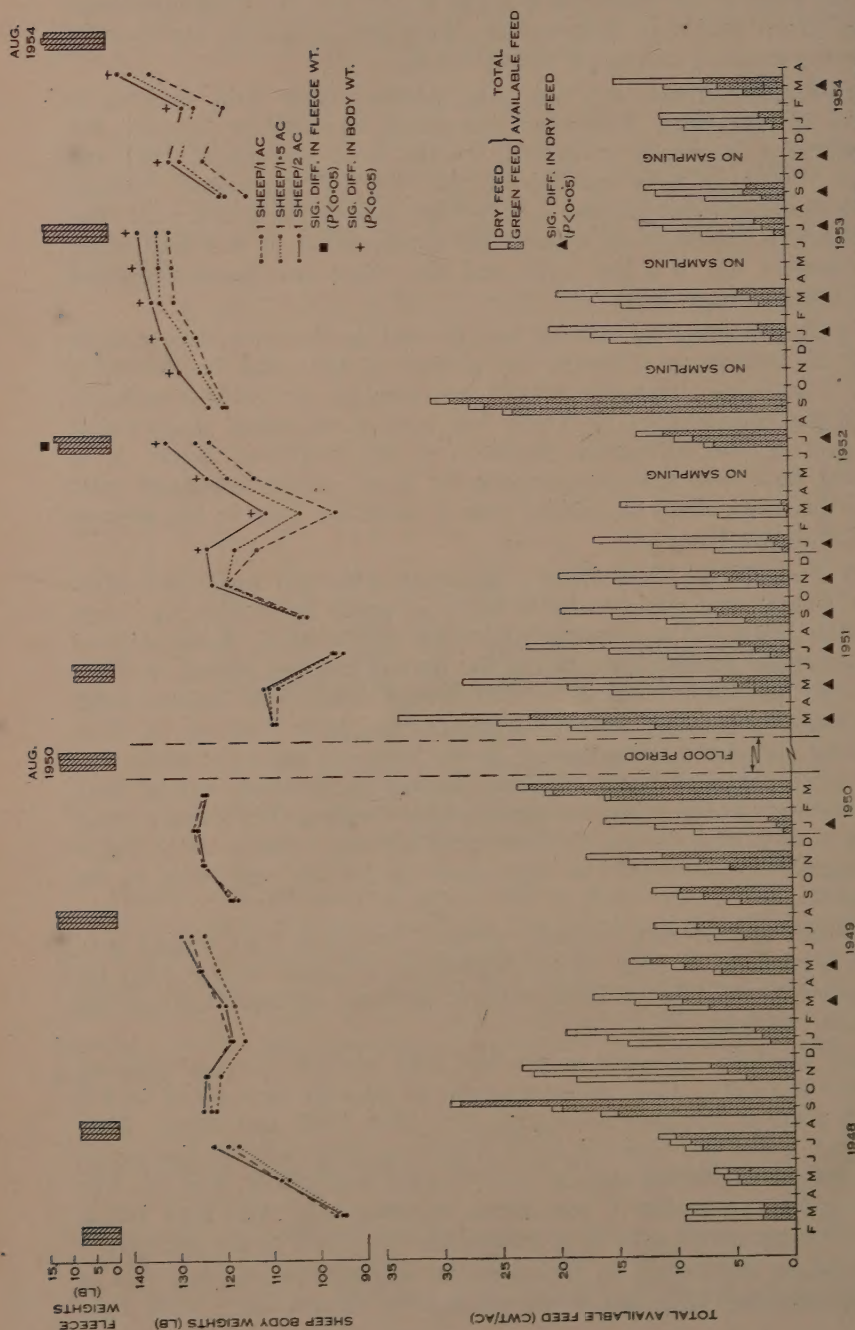


Fig. 2.—Available feed, sheep body weight, and greasy fleece weight at three stocking rates for the periods March 1948–March 1950 and March 1951–March 1954. For each sampling, available feed and fleece weights at 1 sheep/ac, 1 sheep/1.5 ac, and 1 sheep/2 ac are shown in that order from the left-hand column. July–September breaks in the body weight graph correspond to shearing and partial flock replacement (except no replacement in 1948). Significant differences are those between 1 sheep/ac and 1 sheep/2 ac. Differences in green feed were significant at all samplings from September 1948 onwards.

stocking. This effect was probably a reflection of the relative advantage in summer feed. Summer grazing conditions were more favourable in subsequent years and fleece weights were similar despite continuing differences in body weight.

Taken over 5 years (omitting 1948 and 1951 shearing data), light stocking was only slightly superior to heavy stocking in greasy fleece weight, the respective averages being 12.8 and 12.6 lb.

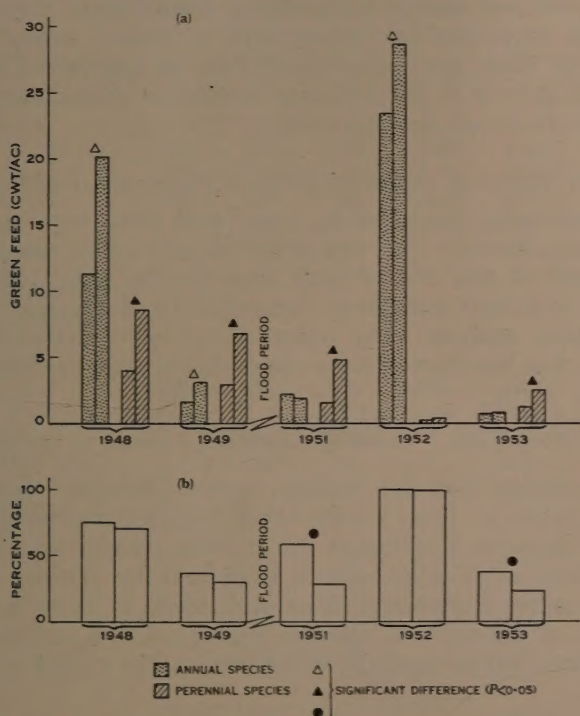


Fig. 3.—Green feed from annual and perennial species at spring (September) sampling in relation to heavy and light stocking, showing: (a) amount of green feed; (b) percentage contribution by annual species to total green feed. For each sampling, values for heavy stocking (1 sheep/ac) are shown by the left-hand column, light stocking (1 sheep/2 ac) by the right-hand column.

(iii) *Botanical Composition of Green Feed.*—Following the driest winters (1951 and 1953) light stocking provided a significantly lower percentage of annuals in the total green feed (Fig. 3). These winters were characterized by a relatively weak growth of annuals on all treatments, which led to concentration of grazing upon perennials. The latter species were favoured by light grazing, and accordingly the percentage contribution by annuals declined. Pronounced grazing of annuals occurred

in wet winters but treatment effect on botanical composition was slight. In the extreme case (1952) the heavy growth of annuals provided almost 100 per cent. of the spring green feed, regardless of stocking rate.

(b) *Effect of Deferment on Available Feed and Animal Production*

Deferments had little effect on available feed, wool production, or body weight, compared with continuous grazing. As a result of autumn deferment, green feed showed a significant improvement only during the near optimum conditions for development of winter annual species in March and July 1952; the improvement was not maintained at maturity of this autumn growth in the following September. Data from deferment treatments are therefore not presented.

(c) *Effect of Stocking Rate on Perennial Grasses*

Data concerning plant density and basal area were analysed for individual years, namely, for two pre-flood and three post-flood years. Values obtained at the end of each experimental year (March) were corrected for treatment differences present at the beginning of the year, using covariance analysis. The index of current differential effect of stocking rate was based on the corrected values, and was defined as the ratio of

$$\{ \text{Mean (1 sheep/2 ac)} - \text{mean (1 sheep/ac)} \} / \text{mean (3 stocking rates)}.$$

It is recognized that this analysis cannot eliminate residual effects from previous seasons. These are the effects of initial treatment differences which, under uniform stocking in the current season, would appear as treatment effects after a covariance analysis. The method is retained because the relative importance of residual effects is likely to be reduced by the factor of competition between perennials. In addition, there is competition from winter annuals, which are usually grazed in preference to perennials.

TABLE 1

DENSITY AND BASAL AREA OF ESTABLISHED PERENNIAL GRASSES UNDER THREE STOCKING RATES* AT MARCH OF EACH YEAR, WITH STOCKING RATE INDICES AND RAINFALL DATA. Values for 1948 and 1951 are as measured; remainder are corrected by covariance analysis for initial differences in the respective years

Quantity	1948	1949	1950	1951†	1952	1953	1954
Density of perennial grass component (plants/sq. m):							
1 sheep/ac	24.6	25.3	24.6	32.5	12.7	36.9‡	22.3
1 sheep/1.5 ac	24.3	25.8	23.2	31.5	12.9	25.4	22.7
1 sheep/2 ac	19.7	23.0	21.3	25.0	12.1	25.2‡	21.6
Mean		24.7	23.0		12.6	29.2	22.2
Index of stocking rate§		-0.09	-0.14		-0.05	-0.40	-0.03

TABLE 1 (Continued)

Quantity	1948	1949	1950	1951	1952	1953	1954
Basal area of perennial grass component (% of total area):							
1 sheep/ac	1.21	1.69†	3.13	2.37	0.76†	2.20	2.11†
1 sheep/1.5 ac	1.17	1.79	3.32	2.66	1.09	1.95	2.36
1 sheep/2 ac	1.20	1.99†	3.51	2.49	1.13†	2.24	2.86†
Mean		1.82	3.32		0.99	2.13	2.44
Index of stocking rate		+0.16	+0.11		+0.37	+0.02	+0.31
Basal area of <i>Chloris acicularis</i> Lindl.:							
1 sheep/ac	0.38	0.59	1.08	0.97	0.47†	1.37	1.48†
1 sheep/1.5 ac	0.37	0.56	1.08	1.16	0.59	1.39	1.81
1 sheep/2 ac	0.45	0.68	1.21	1.35	0.72†	1.50	2.15†
Mean		0.61	1.12		0.59	1.42	1.81
Index of stocking rate		+0.15	+0.12		+0.42	+0.09	+0.37
Basal area of <i>Digitaria coenicola</i> (F. Muell.) Hughes:							
1 sheep/ac	0.15	0.12	0.37	0.64	0.12	0.49	0.40
1 sheep/1.5 ac	0.20	0.13	0.33	0.68	0.15	0.40	0.36
1 sheep/2 ac	0.08	0.13	0.34	0.46	0.15	0.48	0.43
Mean		0.13	0.35		0.14	0.46	0.40
Index of stocking rate		+0.08	-0.09		+0.21	-0.02	+0.08
Basal area of <i>Stipa falcata</i> Hughes:							
1 sheep/ac	0.48	0.70	1.28	0.54	0.14	0.14	0.06
1 sheep/1.5 ac	0.40	0.79	1.32	0.60	0.22	0.12	0.11
1 sheep/2 ac	0.54	0.77	1.30	0.57	0.26	0.08	0.09
Mean		0.75	1.30		0.21	0.11	0.09
Index of stocking rate		+0.09	+0.02		+0.57	-0.55	+0.33
Rainfall (in.):							
March-April		2.88	4.67		1.08	5.18	2.13
Winter		11.48	9.48		8.35	14.47	7.03
Annual		18.46	29.34		11.70	25.89	17.32

* Means for each stocking rate are derived from 72 quadrats during 1948-1950, 54 quadrats during 1951-1954.

† Flood period intervened between March 1950 and March 1951.

‡ Significant difference ($P < 0.05$).

§ Index of current effect of stocking rate (see text) is the ratio

[Mean (1 sheep/2 ac) — mean (1 sheep/ac)]/mean (all stocking rates).

The relation of the stocking rate indices for density and basal area to seasonal rainfall is shown in Tables 1 and 2.

(i) *Density*.—Throughout the experiment the density of the perennial grass component tended to be higher with heavy stocking, the difference

being significant at March 1953 (Table 1). The current differential effect of stocking rate was correlated with winter rainfall and tended to be influenced also by March-April rainfall (Table 2). The relation was such that heavy stocking favoured the greater increase in density during wet winters, and light stocking the least decline during dry winters. The correlation was not significant for density of the main species individually but the yearly responses of each tended to follow the pattern of the whole population.

TABLE 2
CORRELATION COEFFICIENTS (r)* OF RELATIONSHIP BETWEEN STOCKING RATE INDICES AND SEASONAL RAINFALL
Minimum value of r for significance at 5% level (3 degrees of freedom), -0.88

Stocking Rate Indices for	Mar.-Apr. Rainfall	Winter Rainfall (Apr.-Sept.)	Annual Rainfall (Mar.-Feb.)
Density of perennial grass component	-0.81	-0.89	—
Basal area of perennial grass component	-0.96	-0.85	-0.87
Basal area of <i>Chloris acicularis</i>	-0.92	—	-0.85
Basal area of <i>Digitaria coenicola</i>	-0.93	—	-0.99
Basal area of <i>Stipa falcata</i>	-0.92	-0.89	—

* Only the values attaining or approaching statistical significance are shown.

(ii) *Basal Area*.—In contrast with density, the basal area of the perennial component tended to be higher with light stocking. Differences between light and heavy stocking became significant at the end of the first, third, and fifth years of record (Table 1). The index of differential treatment effect was closely correlated with March-April rainfall (Table 2), in that with decreasing rainfall there was a greater difference in favour of light stocking. Similar inverse relationships with both winter and annual rainfall almost reached statistical significance.

It is also evident that stocking rate effects on the three principal species were strongly influenced in the same manner by March-April rainfall. Further, correlations with winter rainfall and annual rainfall were significant respectively for *Stipa falcata* and *Digitaria coenicola*.

(iii) *Size Class Composition and Botanical Composition*.—An index to longer-term change in size class composition of the perennial pasture is shown in Figure 4. At the beginning of the two stages of the experiment, i.e. at March 1948 and March 1951, the percentage of small plants was similar at heavy and light stocking. At the end of these stages (March 1950 and March 1954) the higher percentage of small plants occurred with heavy stocking.

Botanical composition by basal area showed no significant difference due to rate of stocking, *Stipa falcata* dominating all treatments to March 1950 (Fig. 5). From March 1951, following a high mortality of *Stipa* on account of flooding (Biddiscombe, Cuthbertson, and Hutchings 1954), *Chloris acicularis* assumed increasing dominance, again irrespective of rate of stocking.

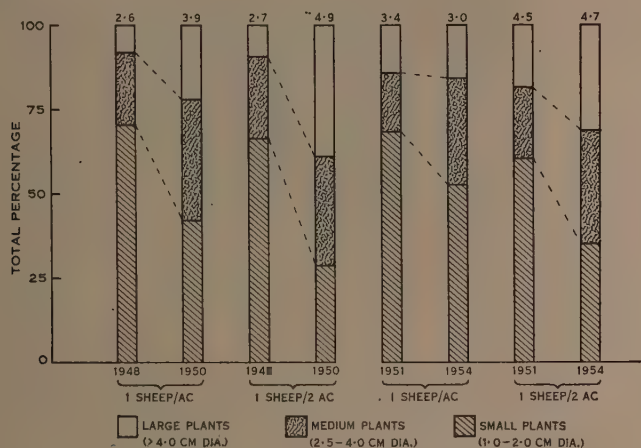


Fig. 4.—Percentage composition of perennial grass component by plant size class at heavy and light stocking, comparing 1948 and 1950 values, and 1951 and 1954 values. Mean plant diameter (cm) at each interval is shown at the top of the column.

(d) Effect of Deferment on Perennial Grasses

Indices of the effects of autumn and spring deferment were compared with rainfall received during deferment, since rainfall for the preceding week did not vary greatly (less than 1 in. on all occasions). The possible influence of post-deferment rain was also examined.

Autumn deferment indices for basal area of *Stipa* tended to be inversely related to rainfall of the deferment period, the correlation being very close to statistical significance (Table 3). No similar trend was evident with respect to the basal area of other species or to the basal area or density of the perennial component, and the data are not presented. The advent of either a wet or dry winter following autumn deferment also appeared to have no influence on treatment effect.

Spring deferment had no significant effect on density or basal area although it was tested under a wide range of rainfall, viz. 0.3-2.9 in. during deferment followed by 2.5-16.4 in. during summer.

VI. DISCUSSION

As a result of generally favourable rainfall adequate feed was available for most of the period, even under heavy stocking. On the other hand at

least three periods of supplementary feeding occurred on heavily stocked properties of the district. The advantage shown by the experimental area may have been due to better pasture utilization on small plots and to almost total exclusion of rabbits over the last 2 years.

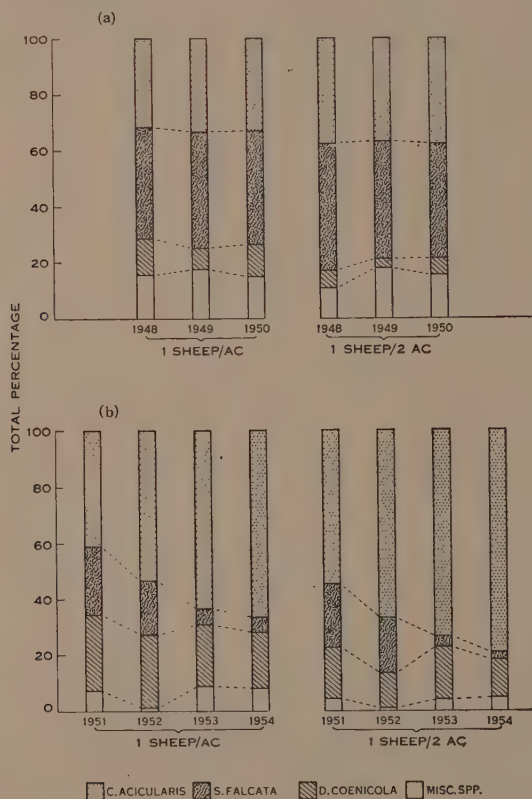


Fig. 5.—Percentage botanical composition of perennial grass component by basal area at heavy and light stocking for the periods 1948-1950 and 1951-1954.

Body weight advantage from light stocking was most pronounced in the driest summer (1951-52) and in turn was traced to the response of the younger animals, i.e. the 1951 replacements. These wethers had not been on a high nutritional plane in the preceding winter and were immature (at 20 months) when entering summer. The group at light stocking received the least growth check and probably were not seriously restricted in muscle or bone development. Autumn recovery of these animals began at the slower rate but the weight level later regained exceeded the level for the heavy stocking group. At light stocking fleece

weight also was least affected by the period of stress. Average fleece weights were comparable for the experimental period as a whole, so that the economic return per acre was greatest from heavy stocking.

The experiment demonstrated the marked influence of March-April rainfall and winter rainfall upon the degree of treatment effect on the density and basal area of various perennial grasses. Annual rainfall was also important in this respect. Generally, light stocking was the most favourable treatment in dry seasons, and heavy stocking in wet seasons (Tables 1 and 2). It is considered that these relationships were mainly

TABLE 3

RELATIONSHIP BETWEEN AUTUMN DEFERMENT EFFECT ON BASAL AREA OF *STIPA FALCATA* AND RAINFALL OF THE DEFERMENT PERIOD

Quantity	Year of Measurement					Correlation Coefficient (r)*
	1949	1950	1952*	1953	1954	
Basal area (% of total area):‡						
Continuous grazing	0.75	1.28	0.09	0.16	0.09	
Autumn deferment	0.76	1.35	0.23	0.11	0.08	
Index§	-0.01	+0.05	+0.88	-0.36	-0.11	
Rainfall of previous deferment period (in.)	1.63	1.79	0.78	3.46	3.50	-0.84

* Flood period intervened between 1950 and 1952.

† Minimum value of r for significance at 5% level (3 degrees of freedom), -0.88.

‡ Values corrected by covariance analysis for initial differences in the respective years.

§ Index of current effect of autumn deferment is the ratio of

[mean (autumn deferment) - mean (continuous grazing)]/mean of both treatments.

due to two factors: (i) direct effect of grazing on the perennial species; (ii) indirect effect through preferential grazing of winter annuals. The perennials carried the brunt of grazing in a dry March-April period, in the presence of weak annuals. In the Trangie environment *Chloris acicularis*, *Digitaria coenicola*, and *Stipa falcata* normally complete a growth cycle during autumn (Biddiscombe, Cuthbertson, and Hutchings 1954) and this late growth stage has been shown by McCarty (1935) to be most important for the storage of food reserves in perennial grasses. It is therefore likely that least defoliation under light stocking allowed greater food storage, and led to better basal area development during the following spring and summer. In a wet March-April period it was observed that grazing was largely transferred to the more vigorous annuals, and perennial grass development under light stocking was probably restricted by greater shading from annuals.

The above-mentioned range of conditions could be expected to affect plant numbers in a similar manner, e.g. in the wet autumn and winter of 1952 it was observed that greater perennial grass regeneration with heavy stocking arose from seedlings which became established in a weaker stand of both perennials (Table 1) and annuals (Fig. 3). These new plants persisted at least until the following March. Spring and summer rainfall were not significant in the relationships but the near drought of October 1951-February 1952 probably increased the advantage of light stocking for the year.

Stipa differed from *Chloris* and *Digitaria* in winter response to stocking rate. This species maintains active growth throughout winter, and under dry conditions the continued close grazing of new shoots evidently depleted its food reserves. Light stocking was favourable in these circumstances, as Roe and Allen (1945) have found for the summer development of *Astrebla* spp. in south-western Queensland. *Chloris* and *Digitaria* were not affected by winter grazing, presumably because these grasses were dormant for most of the period.

Basal area data for the first year of the experiment (Table 1) deserve emphasis. These show that average March-April rainfall and above-average annual rainfall were associated with significant differences between light and heavy stocking. Similarly, only slightly lower March-April rainfall (final year) produced wide treatment differences in *Chloris*, and average winter rainfall (third year) wide differences in *Stipa*.

Annual rainfall of 19 in. and March-April rainfall of 3 in. were arbitrary values taken to separate dry from wet seasons among the 5 years of experiment. On this basis there was a distinct alternation of seasonal rainfall by which heavy stocking effects in a dry year (and March-April period) were counterbalanced by a recovery under favourable rainfall in the following year. The failure of heavy stocking to progressively weaken the perennials is attributed mainly to this incidence of rainfall.

However, examination of long-term records on the above basis suggests that regular alternation of wet and dry seasons is abnormal, e.g. in 39 years of records at Trangie Agricultural Experiment Station the sequence of two consecutive dry seasons predominates for both annual rainfall (16 occurrences) and March-April rainfall (14 occurrences). The significant effects of heavy stocking here established, if carried through the more normal incidence of dry seasons, could hasten the degeneration of perennial pastures shown by Biddiscombe (1953) in his survey of the district. The trend towards a population of small plants under heavy stocking predisposes the pasture to greater damage during dry seasons, since the small plants of these species show the least persistency under dry conditions (Biddiscombe, Cuthbertson, and Hutchings 1954).

Autumn deferment was usually applied from late March or mid April to encourage early development of winter annuals, the treatment taking

no account of growth stage of the perennials. *Stipa* tended to benefit from deferment under dry conditions because the species was capable of continued growth, thus simulating its behaviour with light stocking in a dry winter. Lack of response in *Chloris* and *Digitaria* was probably due to virtual dormancy in these grasses for the latter part of the spelling period. Judging by rate of stocking effects, it is suggested that an earlier deferment (from the first week of March) would benefit these summer-growing perennials, particularly in a dry autumn.

VII. ACKNOWLEDGMENTS

Mr. J. N. Whittet, formerly of the New South Wales Department of Agriculture, and Dr. J. Griffiths Davies, Division of Plant Industry, C.S.I.R.O., originally planned the investigation. The work was directed by a technical committee with members from the Divisions of Plant Industry and Animal Industry of the New South Wales Department of Agriculture, and the Divisions of Plant Industry and of Animal Health and Production, C.S.I.R.O.

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STUDIES IN CITRUS NUTRITION

I. LEAF GROWTH AND COMPOSITION

By R. F. WILLIAMS* and C. T. GATES†

(Manuscript received February 7, 1956)

Summary

Vegetative shoots from the spring flush of an experimental citrus grove were tagged and sampled on three occasions at intervals of 6 months. The effects of four cultural treatments, four levels of nitrogen supply, four combinations of stock and scion, and of time on leaf area and dry weight, and on relative and absolute contents of water, nitrogen, and phosphorus are presented and discussed.

While nitrogen nutrition is still the dominant factor, the evidence strongly suggests that phosphorus nutrition has become important as a determinant of treatment effects within the experimental grove.

The possible relevance of soil temperature and soil organic matter for some of the cultural treatment effects is discussed.

I. INTRODUCTION

Frith (1952) has described a factorial field experiment at Griffith, N.S.W., in which cultural, irrigation, and nitrogen treatments were tested on citrus trees of different stocks and scions. Up to that time, the irrigation treatments had had negligible effects on fruit yield and other attributes measured, but the cultural treatments had had considerable effects, and nitrogen had been effective within the sod treatment. Frith concluded that nitrogen was the overruling factor in the experiment, but indicated that other factors could be operating as well. This view was soon vindicated by the appearance of manganese deficiency in the same experimental grove (Connor 1954). Then too, both Frith and Connor refer to the low phosphorus status of the soils of the area and suggest that the trees were low in phosphorus. They do not imply, however, that phosphorus deficiency was present, and unpublished evidence from another experimental grove on a related soil type had given no yield response to phosphorus. Only when Chapman and Rayner (1951) had established a connection between phosphorus deficiency and poor quality in Washington Navel oranges was it seriously considered that phosphorus deficiency might be an important factor determining fruit quality on the Murrumbidgee Irrigation Areas.

The present study of leaf growth and chemical composition was initiated in 1950 and was intended to cover a wide range of essential elements. The work had, however, to be limited to weight and area changes,

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and to nitrogen and phosphorus. It was soon evident, however, that there was a sufficient association between the leaf phosphorus figures and certain indices of fruit quality to justify an extension of the work. Soil phosphorus studies were therefore made within the experimental grove and, in the hope of establishing the relation on a wider basis, a farm survey for leaf phosphorus and fruit quality was conducted. Leaf growth and composition within the experimental grove is the subject of the present paper, whereas the soil phosphorus studies and the fruit quality survey appear as paper II of this series (Bouma 1956).

The four cultural treatments were:

Tilled (C_1): summer clean cultivation with leguminous (tick bean) green manure crop in winter.

Tilled with manure (C_2): as for C_1 but with 3 tons of farmyard manure per acre per annum.

Sod (C_3): no cultivation, the volunteer weeds mown when necessary.

Bare surface (C_4): no cultivation, weeds controlled by spraying with power kerosene.

Treatments C_1 and C_2 received 1 cwt superphosphate per acre per annum when the cover crop was sown.

The nitrogen treatments were 0, 4, 8, and 16 lb sulphate of ammonia per tree per annum,* in two equal dressings.

The trees in the experiment were planted in 1941 and were of two varieties, Washington Navel and Valencia Late, each variety being budded to Rough Lemon and to Sweet Orange stocks.

Only the heavy and light irrigation treatments were included in this study, and these had no significant effects on the attributes measured.

II. EXPERIMENTAL METHODS

(a) *Sampling Procedure*

The leaf sampling procedure was devised so that valid comparisons could be made in time as well as between treatments and varieties. Vegetative shoots of the 1950 spring flush were tagged in advance of the first sampling date. Tagged shoots were restricted to the northern half of each tree and were approximately at shoulder height. As it was anticipated that leaves from the sod plots would be somewhat smaller than leaves from the other three cultural treatments, 20 shoots were tagged on each tree of the sod plots, but only 17 were tagged in other cases. These numbers allowed for three samplings of 6 and 5 shoots respectively, with 2 spare shoots to cover the contingency of damage to the selected shoots over the rather long period of 12 months. On each sampling occasion, the shoots were taken at random and, from these, 3 centrally attached leaves placed in air-tight containers. Leaves 2, 3, and 4 were taken from

* Referred to hereafter as N_0 , N_1 , N_2 , and N_4 .

shoots with 5 leaves, and leaves 3, 4, and 5 from shoots with 6 or 7 leaves, and so on. Tagged shoots rarely had more than 7 or 8 leaves. The restriction to 3 leaves per shoot was introduced to reduce bias in sampling and because basal, and occasionally terminal, leaves showed abnormalities of form. The procedure thus gave 18 leaves per tree per sampling occasion for the sod plots and 15 leaves for the other cultural treatments. In order to reduce as far as possible the effects of environmental conditions on such quantities as leaf water, sampling was commenced before sunrise when the day had clearly broken, and was completed within 2 hr.

Fresh weights were determined upon arrival at the laboratory and leaf areas were determined by direct comparison with a set of photographic leaf-area standards. These standards were prepared in the manner described by Williams (1954) for tomato leaves and they were placed under a sheet of glass when in use. The samples were dried rapidly in circulating air at 80°.

Preliminary measurements made on a different and more restricted number of tagged shoots had shown that the spring flush did not develop simultaneously on all trees. These differences, however, were confined to the four cultural treatments, there being no effects of the nitrogen and irrigation treatments or of variety. It was estimated that the average dates of attainment of maximum leaf area were October 27 (bare surface) and November 4, 5, and 15 (tilled with manure, tilled, and sod treatments respectively), at which times the leaves were approximately 1 month old. In order to make comparisons between leaves at a comparable stage of development the first samplings were made at a constant time after the attainment of maximum leaf area. The dates were:

Cultural Treatment.	Date (1950)	Delay (days)
Bare surface	Nov. 22	—
Tilled with manure	Nov. 30	8
Tilled	Dec. 1	9
Sod	Dec. 11	19

The remaining two samplings were taken 6 and 12 months later, with no longer any allowance for the differences in time of leaf maturation. The two replicates of the experiment as a whole were sampled on successive days, the second sampling on June 5 and 6, 1951, and the third on December 20 and 21, 1951.

For brevity, the samplings are referred to hereafter as those of Dec. 1950, June 1951, and Dec. 1951. These samples were 2, 8, and 14 months old respectively.

(b) Chemical

Because of the factorial design of the experiment, it was necessary to do the chemical analyses for individual trees, of which 256 were

represented at each sampling. With this coverage it was possible to establish numerous main effects on relative and absolute contents of

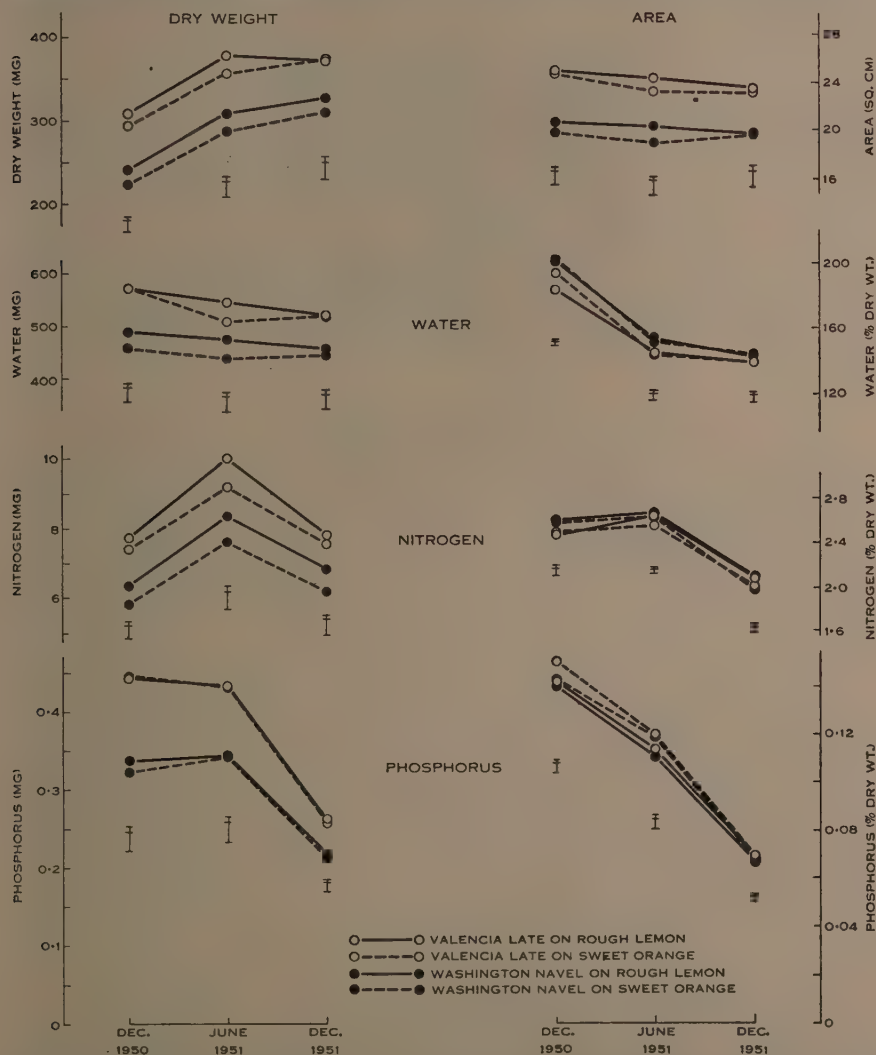


Fig. 1.—The effects of time and of four combinations of stock and scion in citrus on dry weight per leaf, on leaf area, and on absolute and relative amounts of water, nitrogen, and phosphorus in the leaves. Minimum significant differences (1 and 5 per cent. levels) are indicated for comparisons within occasions.

nitrogen and phosphorus with high precision. Significant effects could also be established for the interaction of nitrogen with cultivation treatments.

Nitrogen was estimated by the Kjeldahl method, with selenium as the catalyst, and phosphorus by the colorimetric procedure of Truog and Meyer (1929).

III. PRESENTATION OF DATA

The data are presented almost entirely as text figures, and these have been prepared in such a way that relative effects of treatment can be judged visually for the leaf attributes considered. This was done by adjusting the scales for these attributes so that the general means were made equal in the original drawings.

As there were no significant interactions between variety and treatment, the varietal effects can be examined independently. The relevant data are assembled in Figure 1, which also shows the time trends in the leaf attributes of area, dry weight, water, nitrogen, and phosphorus.

(a) Effects of Time

The time trends of Figure 1 are remarkably similar for all combinations of stock and scion. Leaf area and absolute leaf water show the least change with time, though the small but consistent falls are probably real. The dry weights per leaf show an average increase of 24 per cent. from December to June, but an increase of only 4 per cent. during the next 6 months. Absolute leaf nitrogen increased with dry weight during the first period but there were net losses averaging 19 per cent. during the second period. Absolute phosphorus remained steady during the first period, but net losses averaged 39 per cent. during the second.

Time trends in the relative contents (per cent. dry weight) of water, nitrogen, and phosphorus follow from their absolute values and contemporary trends in the dry weights of the leaves. During the first period, relative contents of water and phosphorus fell with time, but nitrogen did not change. During the second period the water content fell very little, nitrogen fell considerably, and phosphorus fell still more rapidly. Indeed, over the whole period of 12 months, the phosphorus content fell from an average of 0.144 per cent. to the rather low figure of 0.068 per cent.

(b) Effects of Stock and Scion

Leaf size as reflected in area and dry weight is mainly determined by the scion, the leaves of Valencia being more than 20 per cent. larger than those of Washington Navel. Rootstock did have a small, but usually significant, modifying influence on leaf size; the Rough Lemon stock giving on the average a 4 per cent. increase over the Sweet Orange stock.

The small but highly significant effects on relative water content of the leaves are also predominantly due to a scion effect. These are in the opposite direction to those on dry weight, the effects of scion on absolute leaf water being reduced by comparison with those on dry weight.

The effects on relative nitrogen and phosphorus contents are rather small, but some are highly significant. At the first sampling, nitrogen was higher in the leaves of Washington Navels than in the Valencias. By the last harvest, however, this effect had disappeared and was replaced by a rootstock effect of similar magnitude. Leaves on Rough Lemon stock then had a higher nitrogen content. It is not possible at present to suggest explanations for effects such as these, and they do not greatly modify the varietal effects on absolute leaf nitrogen.

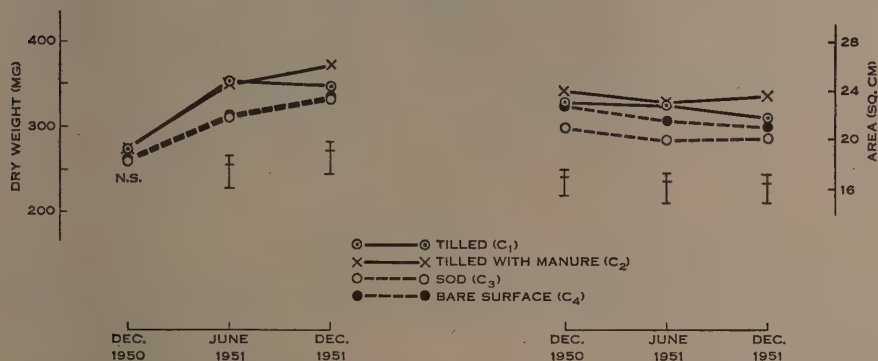


Fig. 2.—The effects of time and of four cultural treatments on dry weight per leaf and leaf area in citrus. Minimum significant differences (1 and 5 per cent. levels) are indicated for comparisons within occasions.

The relative phosphorus contents of the leaves are scarcely affected by the difference in scion, but the values for the Sweet Orange stock tend to be higher than those for the Rough Lemon stock, especially at the June sampling. These effects are opposite in direction and comparable in magnitude to the stock effects on leaf dry weight, and there are no stock effects on absolute leaf phosphorus. The scion effects on absolute phosphorus are, of course, rather large, and are primarily determined by leaf size.

(c) *The Effects of the Cultural Treatments and their Interactions with Nitrogen Supply*

(i) *Dry Weight and Leaf Area.*—The effects of nitrogen level on dry weight and leaf area are small and, for the most part, non-significant. However, the values are consistently low with N₀ and significantly so for dry weight at first sampling.

The main effects of cultural treatment are quite definite (Fig. 2). Both initially and at the June sampling the dry weight per leaf was greater for the two tilled treatments than it was for the sod and bare surface treatments. These effects, though not significant in December 1950, attain or almost attain the 1 per cent. level of significance in the following June. This is also true for the final December sampling for the tilled with manure

treatment. Tillage alone (without manure), on the other hand, gave no increase in leaf weight over the second period of 6 months, so that leaves from this treatment finished with values little greater than those for the sod and bare surface treatments.

In the case of leaf area, the effects of treatment are consistent in time, and the values fall in the order: tilled with manure > tilled > bare surface > sod. The extreme differences are highly significant, and some of the intermediate effects also attain significance.

The differential effects of cultural treatment on leaf area and leaf weight imply differences in dry weight per unit area of leaf. Mean values for the cultural treatments are given as follows:

Treatment	Dec. 1950	June 1951	Dec. 1951
Tilled	1.19	1.57	1.60
Tilled with manure	1.15	1.53	1.59
Sod	1.24	1.58	1.64
Bare surface	1.15	1.47	1.60

At each sampling there is a significant interaction of cultural treatment with nitrogen supply. This is due almost entirely to strong effects of nitrogen within the sod treatment, as will be seen from the following values:

Nitrogen Level	Dec. 1950		June 1951		Dec. 1951	
	Sod	Other Cultural Treatments	Sod	Other Cultural Treatments	Sod	Other Cultural Treatments
N ₀	1.21	1.15	1.51	1.52	1.53	1.60
N ₁	1.20	1.16	1.56	1.51	1.63	1.57
N ₂	1.25	1.17	1.58	1.51	1.71	1.61
N ₄	1.30	1.17	1.68	1.55	1.70	1.61

The extreme values within samplings for the sod treatment differ with high significance and some of the intermediate differences are also significant.

It is possible that the tendency to low values for the bare surface treatment is an expression of the increased shading within the dense canopy of leaves in this treatment. The effect of nitrogen within the sod treatment cannot be explained on this basis.

(ii) *Nitrogen* (Fig. 3).—The cultural treatments and levels of nitrogen have strongly interacting effects on both relative and absolute nitrogen in the leaves. Since the relative values give the better indication of the nitrogen status of the trees, attention is confined to them. The values of Figure 3 show the effects of cultural treatment within each level of nitrogen supply. They could also be presented as effects of nitrogen within cultural treatment and, if this were done, it would be apparent that the nitrogen content was only slightly increased within the two tilled treatments. Within the bare surface treatment, however, the mean values

for N_0 and N_4 were 2.09 and 2.55 per cent. respectively, and within the sod treatment the parallel increase was from 1.94 to 3.01 per cent.

Figure 3 shows that the addition of farmyard manure had no effects on percentage leaf nitrogen, so that the two tilled treatments serve very well as standards of reference for assessing the effects of the other cultural treatments. The sod treatment decreased leaf nitrogen at the N_0 level of supply and also initially at the N_1 level. On the other hand, and

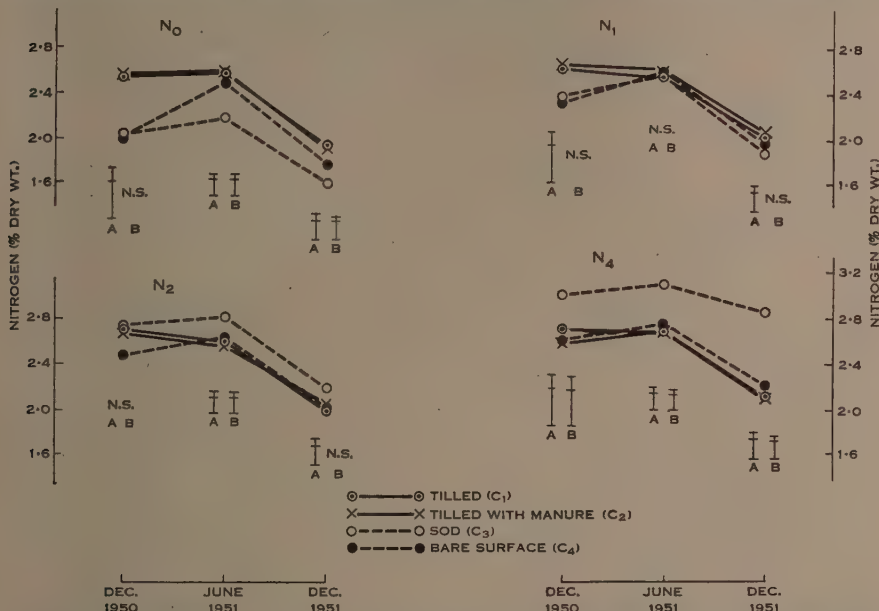


Fig. 3.—The effects of time and of four cultural treatments within four levels of nitrogen supply, N_0 , N_1 , N_2 , and N_4 , on the relative nitrogen content of citrus leaves. Minimum significant differences marked A apply to all cultural-treatment comparisons within occasions except C_1 - C_2 and C_3 - C_4 . Those marked B apply to comparisons C_1 - C_2 and C_3 - C_4 .

with the exception of the first sampling at the N_2 level, the sod treatment increased the nitrogen content with the two higher levels of supply. The effects of the bare surface treatment tended to be intermediate between those of the sod and tilled treatments, though the values for the bare surface treatment never significantly exceeded those for the tilled treatments.

(iii) *Phosphorus* (Fig. 4).—The main effects of the cultural treatments on the relative phosphorus contents of the leaves were highly significant at all samplings. The effect of nitrogen level was also highly significant in June and December 1951, and the two factors gave a significant interaction at the June sampling. The interaction was found to be due almost entirely to a nitrogen effect within the sod treatment.

The sod and bare surface treatments gave very much reduced phosphorus contents at the first sampling, and the values are remarkably low for such young leaves. The reduction remained considerable for the sod treatment at the second and third samplings, but the difference between the bare surface and the tilled treatments was very much reduced for these occasions. The farmyard manure had no effect on the phosphorus content of the leaves. Within the sod treatment, N_4 gave the lowest value at all samplings and in December 1951 there was a progressive decrease from 0.070 per cent. with N_0 to the very low value of 0.047 per cent. with N_4 .

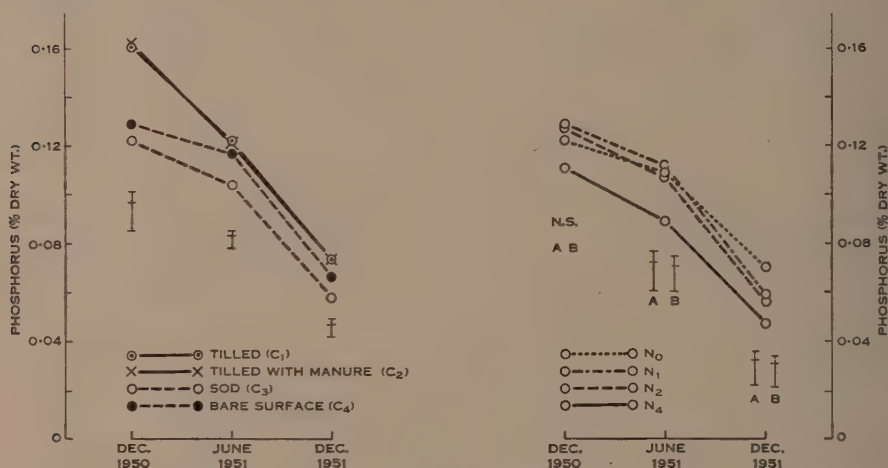


Fig. 4.—The effects of time and of four cultural treatments (left), and of four nitrogen levels within the sod treatment (right), on the relative phosphorus content of citrus leaves. In the latter case minimum significant differences marked *A* apply to nitrogen effects other than N_0 - N_4 and N_1 - N_2 , and those marked *B* apply to comparisons N_0 - N_4 and N_1 - N_2 .

Absolute leaf phosphorus contents (mg/leaf) are of rather less value for diagnostic purposes, but the effects of cultural treatment and time may be summarized as follows:

Treatment	Dec. 1950	June 1951	Dec. 1951
Tilled	0.441	0.432	0.258
Tilled with manure	0.448	0.423	0.280
Sod	0.324	0.326	0.189
Bare surface	0.338	0.369	0.224
Min. sig. diff. ($P < 0.05$)	0.048	0.042	0.029

Net losses of phosphorus sustained by the leaves were similar in all cases, but there were highly significant effects of the cultural treatments at all samplings.

IV. DISCUSSION

Smith and Reuther (1950) studied seasonal changes in the leaves of Valencia oranges on Rough Lemon stock and found leaf dry weight to reach a maximum during the winter and to fall at the time of new growth and bloom in the spring. In the present experiment the same varietal combination showed no change during the same period (Fig. 1) and other varieties increased by 5 per cent. Time trends in relative and absolute nitrogen content were rather similar in the two sets of data, but the phosphorus values fell much more markedly with time in the present experiment. Smith and Reuther claim that the removal of phosphorus from leaves is particularly pronounced during blossom development.

Jones and Parker (1951) also give seasonal trends for leaf nitrogen and phosphorus in trees for which the range of phosphorus content was low enough to affect fruit quality, though not fruit yield. Comparisons for appropriate samplings suggest that the time trends are similar to those of the present experiment, but that the phosphorus contents were, if anything, a trifle lower in the latter.

Chapman and Rayner (1951) grew their experimental trees in large aerated solution cultures and with five levels of phosphorus supply. Only the lowest level gave marked deficiency symptoms but their second level did affect fruit quality. The leaves of the phosphorus-deficient trees showed no very specific symptoms, and the general condition of the trees was somewhat akin to nitrogen deficiency, except that a certain amount of new growth continued. These authors suggest that the phosphorus for this new growth appears to be translocated from older leaves, possibly leading to premature shedding. A somewhat unusual feature of their analyses of spring cycle leaves is that the phosphorus contents increase gradually through the summer to maxima in the winter and then fall. In the present experiment (Fig. 4) the values fall continuously with time, and this is the more usual finding. A plausible explanation is that, under field conditions, the phosphorus-supplying power of the soil may be much higher in the spring following a period of low demand. It is possible, however, that internal factors also operate, for Williams (1948) has pointed out that the redistribution of phosphorus within a plant is facilitated by the relative shortage of nitrogen. Chapman and Rayner's (1951) trees had ample nitrogen, and this may have deprived the spring cycle leaves of an important alternative source of phosphorus for early growth. On this argument the premature shedding of leaves would be greater when both nitrogen and phosphorus were low.

Aldrich and Coony (1952) took for their leaf analyses the most recently matured cycle of growth and proposed 0.07 to 0.10 per cent. as the deficiency range. They quote Chapman and Fullmer (1951) to the effect that vegetative and yield responses may be expected when the phosphorus content of 3- to 7-month-old leaves falls below 0.07 per cent. In

the present experiment the phosphorus content of leaves in their eighth month (June 1951) did not fall below 0.10 per cent. except for the highest level of nitrogen in the sod treatment (mean of 0.089 per cent.). By the 14th month (Dec. 1951), however, the phosphorus contents were very low indeed, the highest being in the tillage treatments with low nitrogen (0.079 per cent. P) and the lowest in sod with high nitrogen (0.047 per cent. P). The range of individual tree values for this treatment was from 0.039 to 0.055 per cent. These extremely low values strengthened the suspicion that phosphorus deficiency was becoming a factor of importance in the experimental grove, and they prompted the seeking of correlations between leaf phosphorus and indices of fruit quality (Bouma 1956). The trees were also examined carefully for the bronzed leaf colour, weak new growth, and abnormal leaf abscission reported by Chapman and Rayner (1951) for phosphorus deficiency in citrus. Such symptoms were undoubtedly present in many trees with low leaf-phosphorus contents, but their diagnostic value was not good, especially where symptoms of nitrogen or manganese deficiency were also present. It has been indicated that the tilled plots received 1 cwt superphosphate per acre when the cover crop was sown. This would account for the high initial values for leaf phosphorus in the tilled treatments (Fig. 4) and for the fact that Whelan (unpublished data) has shown that soil from the tilled treatments had 40 p.p.m. readily soluble phosphorus (Mehlich's method), the sod and bare soil treatments having only 20 p.p.m. readily soluble phosphorus (see Connor 1954). It is not easy to explain the rather small differences in relative leaf-phosphorus values at later harvests for the bare surface treatment (no phosphate additions) and the tilled treatments. The added phosphate may have become unavailable to the trees. While this experiment does not prove it, the indirect evidence strongly suggests that phosphorus deficiency has become an important factor in the experimental grove, and that it may also be important for citrus groves on allied soil types of the Murrumbidgee Irrigation Areas.

The main features of the time trends and treatment effects found by Frith (1952) for relative leaf nitrogen (Navel on Rough Lemon) were confirmed by the present data (all varieties) for the following season (Fig. 3). There seems, however, little likelihood that leaf nitrogen was initially higher in the trees of the bare surface treatment than in those of the tilled treatments, and this point is important for Frith's tentative interpretation of higher yield with the bare surface treatment. Frith's observation that spring growth was a week earlier for the bare surface treatment has been confirmed (see Section II) and the advancement is independent of nitrogen supply. It is now suggested that this advancement could be due to soil temperature differences such as those reported by West (1932) for comparisons of cultivated with bare undisturbed soil. Those studies happen to have been made with a similar soil within a mile of the site of the present experimental grove. The cover crop is turned

under in September and the spring flush begins early in October, and for this time in spring West's data suggest that temperatures at 30 cm (median root depth 28 cm) would be about 2°C higher in undisturbed than in cultivated soil. Furthermore, his data indicate a delay of at least 14 days for temperature in cultivated soil to rise in the spring. Soil temperature differences may thus be an important factor in the timing of the spring flush and hence of the yield advantage in the bare surface treatment. It is also possible that soil temperatures may play some part in the delayed flush growth of the trees of the sod plots, for vegetative cover may further delay the rise of soil temperature in the spring. Johnson and Davies (1927) have shown, for a depth of 1 cm only, that mean maximum temperatures in the spring remain below air temperatures longer under grass than under all other surfaces tested. However, they also found that the minima tend to be higher under grass at all times of the year.

Frith attributes the very high values for leaf nitrogen in the sod, high-nitrogen treatment to the differences in tree size between cultural treatments or to an indirect effect of a supposed relative shortage of phosphorus in the sod treatment. This relative shortage is now well established and is greatest at the highest level of nitrogen. Furthermore, phosphorus deficiency may even be the dominant factor limiting tree size in this treatment (C_3N_4).

Pierre (1948) suggests that in soils low in available phosphorus, the addition of plant materials low in phosphorus but high in nitrogen and energy material may lead to phosphorus starvation of growing plants, and this could be a factor in the cultural treatment effects on leaf phosphorus content. In addition to the direct effect of competition between the weeds and trees of the sod treatment, there are appreciable quantities of organic matter (mowings and other residues) rotting in the surface soil, and their effect on phosphorus availability is likely to be greater the higher the level of nitrogen. The ploughing-in of the cover crop in the tilled treatments may also reduce rather than increase phosphorus availability, though this will depend on the phosphorus content of the beans when turned in. On the bare surface plots, to which no phosphate was added, organic matter additions to the soil would be smallest of all. This treatment has produced the biggest trees and fruit yields, and yet its leaf phosphorus contents at 8 and 14 months are little lower than those of the tilled treatments which had received 1 cwt of superphosphate per annum for 4 years. Other cases in which leaf phosphorus content has been reduced by fertilizing citrus with nitrogen have been reported by Wallace *et al.* (1952) and Smith *et al.* (1954).

The rootstock effect such that leaf phosphorus was higher in trees on Sweet Orange than on Rough Lemon stocks confirms the findings of Smith, Reuther, and Specht (1949) and Aldrich and Buchanan (1954). In the latter case only trees on Rough Lemon rootstock gave phosphorus-deficient symptoms.

V. ACKNOWLEDGMENTS

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STUDIES IN CITRUS NUTRITION

II. PHOSPHORUS DEFICIENCY AND FRUIT QUALITY

By D. BOUMA*

(Manuscript received March 13, 1956)

Summary

The effects of four cultural treatments and four nitrogen levels on fruit quality, percentage leaf phosphorus, and percentage leaf nitrogen in an experimental citrus orchard are presented. In general, the trees in the tilled treatments, which receive some superphosphate to secure a good growth of the winter cover crop, produce fruit of better quality than those in the no-tillage treatments. Increasing levels of nitrogen supply in the latter cause a marked decrease in fruit quality. This effect is associated with a decrease in leaf phosphorus in these treatments. Analyses of soils in the different cultural treatments point also to an unfavourable phosphorus status for the no-tillage treatments. A comparison with earlier results in this experiment indicates that fruit quality, particularly in the no-tillage treatments, is deteriorating. This progressive decline is shown to be related to a deterioration in the phosphorus status of the trees in these treatments.

Highly significant correlations are reported between fruit quality indices and leaf phosphorus content. Less significant correlations were established with readily available soil phosphorus. These correlations were established in a survey of selected orchards in the Murrumbidgee Irrigation Areas, and similar correlations were obtained for the experimental orchard.

I. INTRODUCTION

From a study of leaf growth and composition in an experimental citrus orchard at Griffith, N.S.W., Williams and Gates (1956) concluded that, though nitrogen was still the dominant factor determining treatment effects within the grove, there was strong evidence that phosphorus nutrition had also become an important factor.

Williams and Gates (1956) also suggested an association between leaf phosphorus figures and certain indices of fruit quality within the experimental orchard, although presenting no supporting evidence. The causal relation between phosphorus deficiency and poor quality in Washington Navel oranges has been established in water culture studies by Chapman and Rayner (1951), who fully reviewed the literature relating to this subject.

The present paper examines the bearing of certain cultural and fertilizer practices on the incidence of phosphorus deficiency and on its association with fruit quality. Evidence is also presented to show that phosphorus deficiency is becoming important for citrus growing on the Murrumbidgee Irrigation Areas.

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II. METHODS

In a factorial field experiment with citrus at Griffith, N.S.W., summer clean cultivation, with and without farmyard manure (3 tons/acre annually), was compared with two no-tillage treatments—a sod treatment and a bare surface treatment. The tilled treatments received superphosphate at the rate of 1 cwt/acre per annum, to secure a good growth of the cover crop (tick beans). All cultural treatments were at four nitrogen levels—0, 4, 8, and 16 lb of ammonium sulphate per tree annually. Details of the experiment have been described by Frith (1952).

For the present study, eight Washington Navel trees on Rough Lemon stock were available in each treatment.

(a) Quality Determinations

In July 1954 a sample of two oranges was picked at random from the north side of each of the 128 trees, and brought into the laboratory for the following determinations:

(i) Juice percentage: the weight of the juice as a percentage of the weight of the whole fruit.

(ii) Skin thickness: the oranges were cut in halves equatorially and the skin thickness measured (in millimetres) at four points.

(iii) Sugar percentage of the juice: estimated by the Brix hydrometer.

(iv) Acidity: the amount of 0.1N sodium hydroxide (ml) required to neutralize 10 ml of the juice.

(v) Volume weight: weight per unit volume (g/c.c.). This is a generalized index of quality which is influenced by the percentage of juice by volume, the specific gravity of the juice, and the thickness of the rind. For example, a thin-skinned, juicy, and sweet orange tends to have a high volume weight.

(b) Leaf Analysis

In February 1954, 20 spring cycle leaves were taken from vegetative shoots on the north side of each of the 128 test trees. The samples were dried rapidly at 75°C, ground, and analysed for total nitrogen and phosphorus as described in paper I of this series (Williams and Gates 1956). Results are expressed as percentages of leaf dry matter.

(c) Soil Analysis

In September 1954 the plots were sampled at three depths: 0-4 in., 4-8 in., and 8-12 in. Four samples were taken in each plot and subsequently bulked. After drying and grinding, the soils were analysed for readily available phosphorus according to the method of Truog (1930).

Total phosphorus was estimated in the hydrochloric acid extract, as outlined by Piper (1947) and modified by Hutton (personal communication,

1955). Samples for these analyses were taken in March 1955 at eight sites per plot, and bulked on a plot basis.

In all cases 1-in. cores were taken.

III. RESULTS

(a) Factorial Experiment

(i) *Fruit Quality*.—Frith (1952) presented fruit quality data for this experiment for the years 1949-1951 inclusive, but in greatest detail

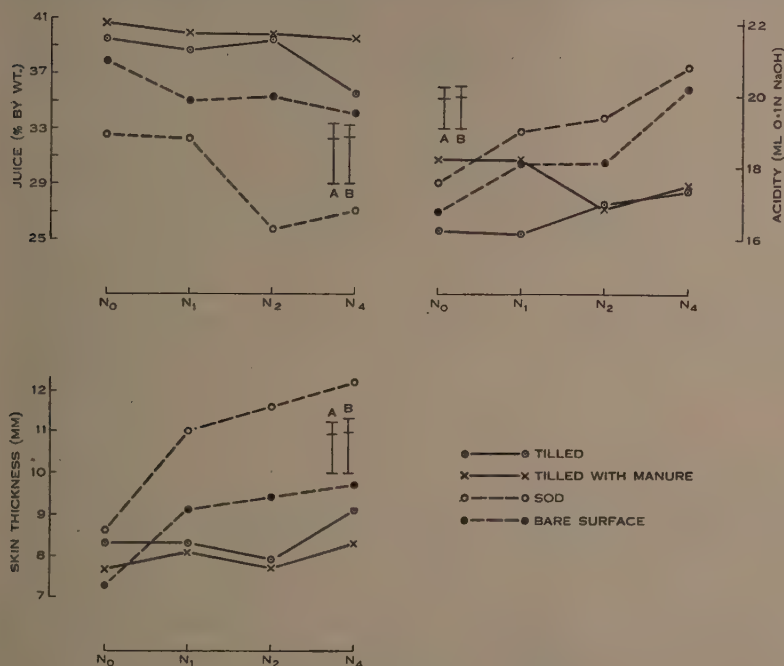


Fig. 1.—Fruit quality attributes as related to cultural treatments, and the interaction of nitrogen levels therewith (N_0 , N_1 , N_2 , and N_4 denote 0, 4, 8, and 16 lb ammonium sulphate per tree per year). Minimum significant differences ($P < 0.05$ and $P < 0.01$) are indicated by vertical lines in this and the next figure. Line A indicates significant differences for comparisons of nitrogen levels within a particular cultural treatment. Line B indicates significant differences for comparisons of cultural treatments within a particular nitrogen level.

for 1950. At that time quality had deteriorated in the sod treatment, the deterioration being greatest for plots receiving ample nitrogen. Fruit from the bare surface treatment was similar in quality to that from the tilled treatments, though juice content and volume weight were slightly but significantly reduced in the former.

A comparison between the different cultural treatments in 1954 (Fig. 1) shows that fruit of poorer quality was still produced in the sod treatment, but that a definite decline in quality in the bare surface treatment had developed since 1950. This was even becoming evident in the sugar contents. Here the nitrogen $\times$ cultural treatment interaction was not significant, and the main cultural effects were:

Treatment:	Tilled	Tilled-with-manure	Sod	Bare surface	Min. sig. diff. ($P < 0.05$)
Sugar content (°Brix):	10.18	10.36	9.63	9.81	0.58

The sugar content of the fruit from the sod and bare surface treatments tended to be lower than in the tilled treatments.

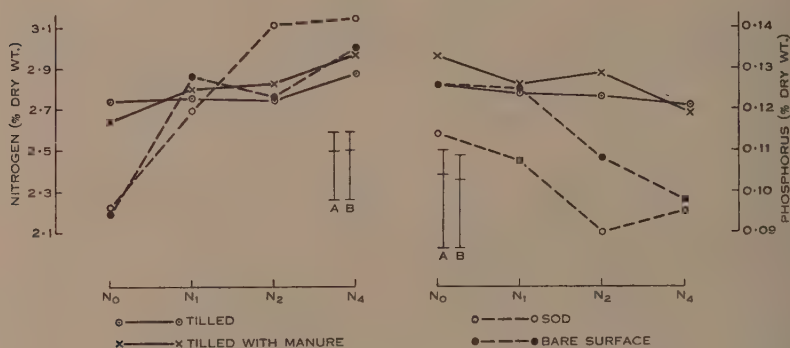


Fig. 2.—Nitrogen and phosphorus contents of leaves in different cultural treatments and the interaction of nitrogen levels therewith (N₀, N₁, N₂, and N₄ denote 0, 4, 8, and 16 lb ammonium sulphate per tree per year).

Of the tilled treatments, the treatment with manure tended to give fruit of slightly better quality than the treatment without manure.

The deterioration of fruit quality with higher nitrogen levels is still very pronounced in the sod treatment, but has now (1955) also developed in the bare surface treatment. Even in the tilled-without-manure treatment, the juice content is significantly reduced at the highest nitrogen level.

(ii) *Nitrogen and Phosphorus Status of the Trees.*—Nitrogen and phosphorus contents of spring flush leaves are presented in Figure 2.

The nitrogen contents in the sod and bare surface treatments without nitrogen, were very much lower than in the tilled treatments. The lowest nitrogen application in the no-tillage treatments resulted in a sharp increase in nitrogen content. While higher nitrogen levels continued to increase the nitrogen content in the sod treatment, no further increase occurred in the bare surface treatment. In this treatment the nitrogen

content remained at approximately the same level as in the tilled treatments.

The early occurrence of severe nitrogen deficiency in the sod treatment was pointed out by Frith (1952). Since then, rather severe deficiency symptoms have also developed in the bare surface treatment, and soil nitrogen would appear to be almost exhausted. However, in the tilled-no-nitrogen treatments, nitrogen is still available in sufficient quantities to maintain healthy tree growth. In these cases it is likely that nitrogen is being released by the breakdown of organic matter under cultivation, and that the organic reserves are being maintained to some extent by the leguminous cover crop.

The phosphorus contents in the leaves of the no-tillage treatments showed significant decreases with increasing nitrogen levels. In the bare surface-without-nitrogen treatment, the phosphorus content was approximately the same as in the tilled-no-nitrogen treatments (0.126 per cent.), but fell to the low value of 0.096 per cent. with the highest nitrogen level. In the sod treatment the same trend was apparent, but the phosphorus contents were always lower than for the bare surface treatment.

An inverse relation between level of nitrogen supply and leaf phosphorus was present for the sod treatment as early as 1951 (Williams and Gates 1956), and was best developed in the oldest leaf sample. The same relation is now evident in both of the no-tillage treatments. These effects are difficult to explain without further data.

The concurrence of fruit quality decline, and the deterioration of the phosphorus status of the trees in the no-tillage treatments since 1950, will be apparent. A possible connexion between fruit quality and phosphorus nutrition is also suggested by a comparison of Figures 1 and 2. Quality indices in all treatments follow the phosphorus contents rather closely. In the tilled treatments the phosphorus status is better than in the no-tillage treatments, and quality in the former has remained reasonably good. In the no-tillage treatments the phosphorus contents decrease with increasing nitrogen levels, and this is accompanied by a decline in quality. These findings are of significance in view of the establishment of a causal relation between phosphorus deficiency and poor fruit quality in citrus by Chapman and Rayner (1951). This relation is discussed further in Section IV.

(iii) *Phosphorus Status of the Soil.*—In view of the differences found in leaf phosphorus between the cultural treatments, it was decided to analyse soil samples for readily available, and total, soil phosphorus (Table 1). Only the main cultural effects are given because the nitrogen $\times$ cultural treatment interactions were not significant.

For the 0.4 in. layer, the tilled-without-manure treatment showed at least six times the amount of readily available phosphorus as compared with the no-tillage treatments. This increase was doubtless due to the application of superphosphate for the cover crop and perhaps also to the

summer cultivation. When manure was also applied a further large increase resulted. The increases were significant at all depths. The effect of farmyard manure was detectable even in the 8-12 in. layer, where there was nearly twice as much readily available phosphorus as in the treatment without manure. The effect of superphosphate and farmyard manure was also evident in the increases in total phosphorus. These increases were mainly confined to the top soil, and at 8-12 in. depth the differences between the cultural treatments were barely significant.

TABLE 1
READILY AVAILABLE AND TOTAL SOIL PHOSPHORUS

Depth	Tilled		Not Tilled		Minimum Significant Difference ($P < 0.05$)
	Without Manure	With Manure	Grass Sod	Bare Surface	
<i>Available phosphorus (p.p.m.)</i>					
0-4 in.	34.2 (1.5310)*	53.7 (1.7201)	4.4 (0.6275)	6.6 (0.8110)	(0.0662)
4-8 in.	15.8 (1.1696)	28.1 (1.4285)	3.8 (0.5468)	4.3 (0.6187)	(0.1017)
8-12 in.	5.9 (0.7540)	10.0 (0.9562)	4.0 (0.5285)	4.4 (0.6465)	(0.0728)
<i>Total phosphorus (p.p.m.)</i>					
0-4 in.	220	259	155	160	13
8-12 in.	146	144	137	134	12

* Figures in brackets are the means of the data transformed on a logarithmic basis.

Readily available phosphorus in the top layer of the sod treatment was significantly lower than in the bare surface treatment. At greater depths the difference was barely significant. It is likely that the weeds and organic matter in the top soil of the sod treatment "lock up" part of the little phosphorus available for plant growth.

The experiment was planted on virgin soil, and no superphosphate has been applied to the no-tillage treatments. The low phosphorus status of this soil is evident from Table 1, and the differences between tilled and no-tillage treatments are reflected in the phosphorus status of the trees. The progressive deterioration of the phosphorus nutrition in the no-tillage treatments is probably caused by a gradual exhaustion of the original phosphorus supply, and the earlier occurrence of phosphorus starvation in the sod treatment could be due, at least in part, to the competitive effect of the weeds in this treatment.

(b) Survey of Orchards

(i) *Introduction.*—The results presented so far gave reason to suspect that the deterioration of fruit quality in the Murrumbidgee Irrigation Areas could be due, at least in part, to phosphorus deficiency, possibly aggravated by the continuous use of sulphate of ammonia as nitrogen

source. It was therefore decided to seek confirmation by making a survey of selected farmers' groves.

In November 1954, 26 orchards, free from root rot, salt troubles, or other obvious faults, were selected. For each orchard, questionnaires were filled in, giving information relating to tree health, fertilizer practice, and soil conditions. From each orchard, six of the healthiest trees were selected as close together as possible. All trees sampled were Valencia Late on Rough Lemon rootstock and they were approximately of the same age.

Six spring cycle leaves were taken at random from the north side of each tree, and the six leaf samples were bulked before analysis. Six fruits per tree were sampled, also from the north side. Soil samples were taken at six sites in each orchard, close to the chosen trees, and bulked for analysis. Other analytical methods were the same as for the factorial field experiment.

(ii) *Results.*—In a number of cases deficiency symptoms were noticed, resembling those described for phosphorus deficiency. The leaves showed bronzing and sometimes definite necrotic spots. Some dieback also occurred.

In the majority of cases ammonium sulphate is the only fertilizer at present used. The use of superphosphate was abandoned about 1940 owing to lack of evidence for marked responses and also because of wartime shortages. Almost invariably, good quality oranges are produced where a mixed fertilizer is used. Even on soil types not regarded as suitable for citrus, good quality oranges are produced where superphosphate is used.

The range of phosphorus contents of leaf samples in this survey was 0.096-0.164 per cent. (mean 0.123 per cent.). The values found in the factorial field experiment were in general lower than the mean of this survey.

Readily available soil phosphorus in this survey showed the following values:

0-4 in.:	10-131 p.p.m. (mean 70 p.p.m.),
4-8 in.:	5-71 p.p.m. (mean 38 p.p.m.),
8-12 in.:	3-33 p.p.m. (mean 18 p.p.m.).

In all cases readily available soil phosphorus in the factorial field experiment were lower than these means (Table 1).

Total soil phosphorus values were:

0-4 in.:	246-531 p.p.m. (mean 340 p.p.m.),
4-8 in.:	190-405 p.p.m. (mean 270 p.p.m.),
8-12 in.:	144-291 p.p.m. (mean 202 p.p.m.).

This also points to a low phosphorus status in the factorial field experiment. Even in the tilled treatments, readily available and total phosphorus are considerably below what can be considered as an average for the area.

Correlation coefficients (r) between quality attributes and phosphorus in leaves and soil are presented in Table 2.

All quality attributes are significantly correlated with leaf phosphorus contents. In addition, some significant correlations were found between quality attributes and readily available soil phosphorus. No significant correlations could be established with total soil phosphorus.

TABLE 2
CORRELATION COEFFICIENTS (r) BETWEEN QUALITY ATTRIBUTES AND PHOSPHORUS
CONTENTS OF LEAVES AND SOIL

Quality Attribute	Phosphorus in Leaf Dry Matter (%)	Readily Available Soil Phosphorus at		
		0-4 In.	4-8 In.	8-12 In.
Rind thickness	-0.624***	-0.485*	-0.274	-0.128
Acid content	-0.619***	-0.144	+0.039	+0.011
Volume weight	+0.596***	+0.523*	+0.603**	+0.510*
Juice content	+0.528**	+0.494*	+0.408*	+0.238

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

IV. GENERAL DISCUSSION

In establishing the causal relationship between phosphorus deficiency and poor fruit quality in citrus, Chapman and Rayner (1951) showed that phosphorus deficiency was associated with thick rinds, high acid content,

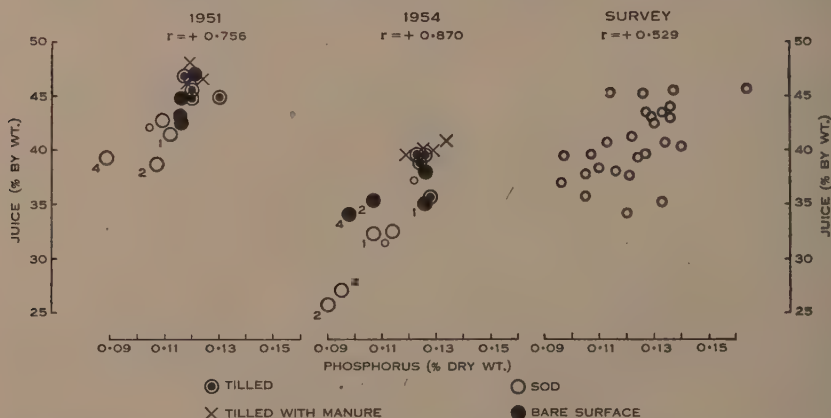


Fig. 3.—Correlations between juice content and leaf phosphorus for the factorial field experiment in 1951 (means of all varieties), for 1954 (Washington Navel on Rough Lemon stock), and for the survey of sample orchards (Valencia on Rough Lemon stock). Nitrogen levels are indicated by 0, 1, 2, and 4, corresponding to 0, 4, 8, and 16 lb of ammonium sulphate per tree per year.

and low juice content, but sugar content was not affected. Evidence on the quantitative relation between leaf or soil phosphorus and the relevant indices of fruit quality is not extensive, however, and the highly significant

correlations of the present paper suggest that the seeking of such correlations may be a useful first step in identifying phosphorus deficiency as a causal factor in a given set of circumstances. Aldrich and Buchanan (1954) found the incidence of phosphorus deficiency in citrus to be associated with low total phosphorus in the soil but not with water-soluble or acid-soluble phosphorus. Water-soluble phosphorus has not been determined, but our present experiments seem to indicate the reverse of these findings. It is with leaf phosphorus, however, that the best correlations were obtained, and some of these relations are shown diagrammatically in Figures 3 and 4.

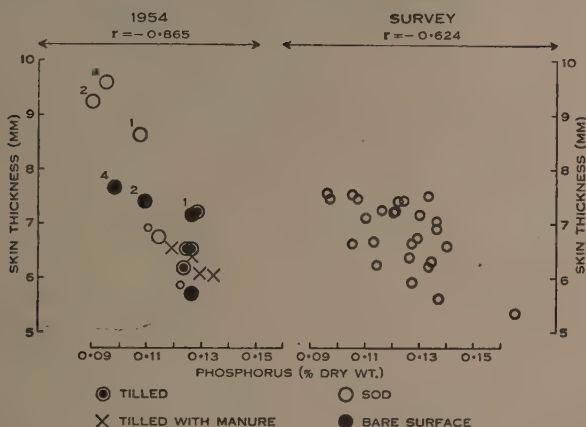


Fig. 4.—Correlations between skin thickness and leaf phosphorus for the factorial field experiment in 1954 (Washington Navel on Rough Lemon stock) and the survey of sample orchards (Valencia on Rough Lemon stock). Nitrogen levels are indicated by 0, 1, 2, and 4, corresponding to 0, 4, 8, and 16 lb of ammonium sulphate per tree per year.

It has also been possible to show a strong relation between juice percentage or acidity and leaf phosphorus for an earlier stage in the life of the experimental grove. The following correlation coefficients were computed from the primary data of Frith (1952) for fruit quality in 1951 (the fourth year of treatment), and of Williams and Gates (1956) for leaf phosphorus in June 1951.

Juice percentage—leaf phosphorus	$r = 0.756$ ($P < 0.001$)
Acidity—leaf phosphorus	$r = 0.748$ ($P < 0.001$)

The relation for juice percentage is included in Figure 3. It will be apparent that the range of juice percentage has increased from 1951 to 1954 and this is an expression of the progressive deterioration of fruit quality in the orchard. Similarly it will be seen that the ranges of juice percentage (Fig. 3) and skin thickness (Fig. 4) are rather less for the

sample orchards than for the experimental grove. These differences doubtless contribute to the smaller numerical values of the correlation coefficients for the sample orchards. Nevertheless, one would expect to encounter a wide range of modifying influences on the sample orchards, and it is a matter for some surprise that the coefficients are so highly significant. Incidentally, it should be remembered that the leaf phosphorus values of Figures 3 and 4 are not for leaves of comparable age.

The rather wide ranges for the quality indices within the experimental grove are due to quality deterioration first in the grass sod treatment and later in the bare soil treatment. Quality has remained fairly good in the tillage treatments, and within these there is a suggestion that farmyard manure is beginning to have a beneficial effect.

Williams and Gates (1956) express the view that their high initial values for leaf phosphorus in the tilled treatments are a reflection of the higher, but still rather inadequate, levels of readily soluble phosphorus in the soil. Internal factors have modified this simple relation for their later sampling occasions, and this seems to apply also for the leaf sample taken in February 1954 (Fig. 2). Phosphorus contents for this sample at 4 months are rather similar to those at 8 months in 1951 and the level of nitrogen supply now has a greater effect within the no-tillage treatments. This effect is very pronounced in the sod treatment. The reason for this nitrogen effect is still rather obscure.

It does not seem profitable to attempt to indicate deficiency levels for leaf phosphorus in citrus, for we still know comparatively little about the effects of age and other modifying factors on this quantity. If, as has been found for the Murrumbidgee Irrigation Areas, there is a strong correlation between leaf phosphorus content and one or more attributes of fruit quality, the next step is to test the association experimentally on a number of groves throughout the district. Small-scale experiments have been laid down on farmers' groves, and there is already some evidence that the application of superphosphate is improving fruit quality.

While it is undoubtedly true that leaf phosphorus content is largely dependent on readily available soil phosphorus, the methods for determining this phosphorus still leave much to be desired, and the present evidence suggests that soil phosphorus studies are likely to be of secondary importance for diagnostic purposes.

Since the work reported here was carried out, a heavy dressing of superphosphate has been applied throughout the experimental grove, and graduated applications of lime have been made to counteract the acidifying effects of the sulphate of ammonia treatments. It will take some years to assess the effects of this change of programme, but studies of leaf composition and fruit quality should yield results of great interest.

V. ACKNOWLEDGMENTS

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THE INHERITANCE OF IMMUNITY TO PEA MOSAIC VIRUS

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Summary

F₁, F₂, F₃, and backcross data from pea crosses between two mosaic-susceptible varieties and two immune varieties indicate that the immune condition is controlled by a single recessive gene, with the proposed designation *mo*.

There is evidence from these data, and a further test of F₁, to suggest that the susceptible heterozygote can be separated from the homozygote (*mo mo*) by: (i) Timing of the appearance of the mosaic symptom after inoculation; (ii) The type of symptom, three being described. The latter, however, appears to depend on the variety, or the parentage in the case of crosses, and the strain or isolate of the virus. These differential susceptibilities are discussed.

I. INTRODUCTION

Pea mosaic virus was recorded in New Zealand by Chamberlain (1936), who later listed immune and susceptible varieties of garden and field peas (Chamberlain 1939). Breeding work, begun in 1931 for the agronomic improvement of the garden variety Greenfeast (Calder 1944), was modified to include the transference of mosaic immunity from known immune varieties. Examination of the breeding records showed that almost all of the immune selections made in F₄ from crosses between immune and susceptible varieties proved to be immune in F₅ and F₆, suggesting a simple mode of inheritance. This paper describes experiments to ascertain the inheritance of immunity.

II. MATERIALS AND METHODS

(a) Test Crosses

Using pure lines of each variety, the following reciprocal crosses were made for study:

Immune Parent	Susceptible Parent	Number of Pure Lines Crossed
William Massey (syn. Kelvedon Wonder)	Blue Prussian	3
William Massey	Greenfeast (syn. Lincoln)	3
Onward	Greenfeast	4
Onward	Blue Prussian	4

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F_1 and F_2 plants were grown on to provide seed for the testing of F_2 and F_3 respectively. The parent variety lines were increased with each generation, and used as controls in all inoculation tests.

(b) Tests for Immunity

Plants were grown in a glass-house in steam-disinfected potting soil, two or three to a 4-in. pot.

Preliminary trials showed that 100 per cent. infection could be obtained in susceptible pea varieties by using inoculum extracted from young leaves of infected plants when vein clearing had become pronounced, and by diluting this with nine parts of water. Plants were inoculated, when the first three leaves were well expanded, by leaf rubbing using as abrasives either powdered carborundum sprinkled over the leaves or the diatomaceous earth, "Dicalite", mixed with the inoculum.

Two isolates of the pea mosaic virus were used. The first was lost after testing all the F_1 crosses and the F_2 of the cross Onward $\times$ Blue Prussian, and was replaced by a strain giving slightly more pronounced symptoms on Greenfeast and causing death in Blue Prussian by systemic necrosis which developed 2 or 3 weeks after inoculation. Both strains were similar to that used by Chamberlain (1939) in their reaction on immune and susceptible pea varieties but differed in that they were able to infect French bean. In this latter respect they were similar to the strain recovered by Fry (1952) from gladioli.

III. RESULTS

On inoculation there were no differences between reciprocals in any crosses in the proportion of plants infected, so in the following results data for reciprocal crosses have been pooled. The lines within varieties showed no differences as controls or in crosses.

All plants of parent lines tested at the same time as the appropriate F_1 , F_2 , F_3 , or backcross progeny reacted to inoculation as expected, showing that the virus strains used remained constant in their effect.

(a) F_1 and F_2 Generations

In F_1 tests, all plants in the four crosses were susceptible and Table 1 shows that F_2 segregations diseased: healthy were reasonably consistent with a 3:1 ratio.

Owing to the large number of plants involved, all crosses were not tested simultaneously. The F_2 of the cross Onward $\times$ Blue Prussian was tested first. A proportion of these plants developed symptoms together, approximately a week after inoculation. The remaining plants to show symptoms did so at irregular intervals. Therefore on testing the remaining three crosses, daily observations on each plant were recorded. The number

of plants of the F_2 which showed mosaic symptoms at 7 days tended to equal the number which remained permanently free of infection.

TABLE 1
 F_2 DISTRIBUTION FROM CROSSES BETWEEN SUSCEPTIBLE AND IMMUNE VARIETIES

Crosses	Total Plants	F_2 Plants		χ^2 (3:1)	P
		Diseased	Healthy		
William Massey × Blue Prussian	233	172	61	0.17	0.68
William Massey × Greenfeast	150	107	43	1.08	0.30
Onward × Blue Prussian	317	255	62	5.01	0.02
Onward × Greenfeast	83	63	20	0.04	0.85
Total	783	597	186	0.65	0.42

Thus on classifying the F_2 data on the basis of plants showing symptoms at 7 days, after 7 days, and those remaining free of symptoms, a correspondence to a 1:2:1 ratio is obtained (Table 2). This suggests

TABLE 2
 F_2 DISTRIBUTION OF DATA FROM THREE CROSSES IN TABLE 1 BASED ON TIMING OF
APPEARANCE OF MOSAIC SYMPTOM
The numbers which died in each class are shown in parentheses

Crosses	F_2 Plants			χ^2 (1:2:1)	P
	Mosaic Symptoms at 7 Days	Mosaic Symptoms after 7 Days	Healthy		
William Massey × Blue Prussian	60(50)	112 (5)	61(1)	0.36	0.84
William Massey × Greenfeast	29(20)	78(22)	43(4)	2.85	0.24
Onward × Greenfeast	17 (4)	46(16)	20(3)	1.19	0.55
Total	106(76)	236(43)	124(7)	1.47	0.48

that among susceptible F_2 plants resulting from a cross between immune and susceptible parents, homozygous plants can be separated from heterozygous by the earliness of expression of the mosaic symptom. Almost all plants of the susceptible parents developed symptoms within 7 days of inoculation.

As the plants approached flowering it was noted that many which had shown mosaic symptoms developed a systemic necrosis which resulted

in death before seed formation. In the two crosses involving William Massey a high proportion of necrotic plants had shown mosaic at 7 days, whilst in the Onward $\times$ Greenfeast cross, plant deaths were more evenly distributed through the three classes. These figures are included in Table 2. The untimed F_2 , Onward $\times$ Blue Prussian, showed negligible mortality, but here the virus isolate used was that which did not cause death in Blue Prussian.

TABLE 3
TYPES OF F_2 FAMILIES BASED ON F_3 BEHAVIOUR

Crosses	Types of Families	No. of Families		Total Individuals		χ^2 (3:1)	P
		Ob- served	Ex- pected	Dis- eased	Healthy		
William Massey $\times$ Blue Prussian	Healthy	7	7		84		
	1H:3D	18	14	111	45	1.23	0.27
	Diseased	3	7	33			
	Total	28	28				
	χ^2 (1:2:1) = 3.43 $P = 0.18$						
William Massey $\times$ Greenfeast	Healthy	13	13		156		
	1H:3D	37	26	394	147	1.36	0.24
	Diseased	2	13	24			
	Total	52	52				
	χ^2 (1:2:1) = 13.96 $P < 0.001$						
Onward $\times$ Blue Prussian	Healthy	14	13.75		168		
	1H:3D	28	27.50	220	72	0.02	0.89
	Diseased	13	13.75	139			
	Total	55	55.00				
	χ^2 (1:2:1) = 0.05 $P = 0.98$						
Onward $\times$ Greenfeast	Healthy	8	8		109		
	1H:3D	16	16	146	45	0.21	0.64
	Diseased	8	8	70			
	Total	32	32				
	χ^2 (1:2:1) = 0.00 $P = 1.00$						

In saving seed for the F_3 progeny test of F_2 , it was to be expected, if a high proportion of homozygous susceptible plants had died in the William Massey crosses, that a close correspondence to the family ratio of 1 non-segregating susceptible:2 segregating susceptible:1 immune would not be obtained.

(b) F_3 Generation

In the crosses Onward $\times$ Blue Prussian and Onward $\times$ Greenfeast, the F_3 families showed a distribution of 1:2:1, confirming F_2 observations. The crosses William Massey $\times$ Blue Prussian and William Massey $\times$ Greenfeast showed a deficit in the number of families in the homozygous dominant class, as expected. Table 3 gives the summarized data of the four crosses.

(c) Backcross Generation

Owing to the inconsistency of F_3 data with F_2 in the William Massey crosses and poor correspondence with a 3:1 ratio in F_2 of the Onward $\times$ Blue Prussian cross, backcrosses of all four F_1 's to corresponding immune parents were tested. Table 4 shows that in all crosses the backcross generation shows correspondence to a 1 susceptible:1 immune ratio.

TABLE 4
DISTRIBUTION FROM BACKCROSSES BETWEEN F_1 AND IMMUNE PARENTS

Crosses	Immune Backcross Parent	Plants from Backcross		χ^2 (1:1)	P
		Diseased	Healthy		
William Massey $\times$ Blue Prussian	William Massey	52	58	0.33	0.57
William Massey $\times$ Greenfeast	William Massey	89	112	2.63	0.10
Onward $\times$ Blue Prussian	Onward	194	194	0.00	1.00
Onward $\times$ Greenfeast	Onward	36	35	0.01	0.90
Total		371	399	1.02	0.31

The symbols *Mo* and *mo* are proposed giving the following genotypes and their reaction to pea mosaic virus; *Mo Mo*, susceptible; *Mo mo*, susceptible; *mo mo*, immune.

(d) Recognition of Heterozygotes

(i) *Time of Symptom Appearance from Inoculation.*—The efficacy of the arbitrary separation of *Mo Mo* and *Mo mo* on the time of appearance of virus symptoms in F_2 as indicated by F_3 segregations is tabulated in Table 5. F_2 plants which had sufficient seed to test F_3 families are shown according to whether symptoms developed at 7, at 8, or after 8 days. Some of the plants which developed symptoms early proved to be heterozygotes while almost all of the plants which developed symptoms late were heterozygotes.

TABLE 5
EFFICIENCY OF SEPARATION OF $M_0 M_0$ AND $M_0 m_0$ IN F_2 AS INDICATED BY F_1 PROGENY TEST*

Crosses	F ₂ Parent Plants Showing Mosaic at 7 Days				F ₂ Parent Plants Showing Mosaic at 8 Days				F ₂ Parent Plants Showing Mosaic after 8 Days			
	Number		Segregating <i>Mo Mo</i>	Total	Number		Segregating <i>Mo mo</i>	Total	Number		Segregating <i>Mo mo</i>	Total
	Homozygous <i>Mo Mo</i>	Homozygous <i>Mo Mo</i>			Homozygous <i>Mo Mo</i>	Homozygous <i>Mo Mo</i>						
Wm. Massey × Blue Prussian	4	2	2	2	—	—	2	2	15	1	14	
Wm. Massey × Greenfeast	5	2	3	1	—	—	1	33	—	—	33	
Onward × Greenfeast	6	4	2	7	2	2	5	11	2	2	9	

* F_3 families of eight or more plants.

TABLE 6
TIME OF APPEARANCE OF MOSAIC SYMPTOMS IN HOMOZYGOUS AND HETEROZYGOUS PLANTS

[illegible]

A further inoculation trial of F_1 crosses was performed to compare the time of symptom appearance in known heterozygotes against susceptible homozygotes. All plants were randomized prior to inoculation to avoid possible environmental effects on any one group or genotype. As Table 6 shows, the mode for time of symptom development in the *Mo mo* genotypes in all crosses tends to be delayed in comparison with the *Mo Mo* genotypes.

Of the pure-line susceptible plants in Table 6, 99 per cent. had developed symptoms 7 days after inoculation while in the homozygous susceptible class in the F_2 (Table 5) only 8 out of 13 showed symptoms after this time interval. Among heterozygotes, less than 3 per cent. of F_1 but 20 per cent of F_2 plants developed symptoms before the ninth day. These results show that the distinction between homozygotes and heterozygotes was more clear-cut when susceptible parents were compared with F_1 heterozygotes than when the two genotypes were compared in the F_2 generation.

Since these tests were not carried out at the same time it is not clear whether the differences are due to a seasonal effect or to variation in the time of symptom development being an expression of hybrid vigour in the F_1 . There were no obvious growth differences between parent lines and F_1 crosses that could be attributed to hybrid vigour.

(ii) *Symptom Differences.*—Throughout the trials symptom differences were apparent among the progeny from different crosses. The differences were confirmed in the final F_1 crosses and the following three categories noted.

- (1) Early mottling followed by systemic necrosis causing death of plants (Fig. 1). The Blue Prussian-*Mo Mo* genotype typifies this condition, paralleled by the suggested *Mo Mo* plants in F_2 of crosses involving William Massey (*mo mo*).
- (2) Mottling and stunting (Fig. 2). Typical of Greenfeast (*Mo Mo*). Seed production is usually restricted.
- (3) Mottling with no other noticeable symptoms (Fig. 1). Seed produced. Heterozygotes *Mo mo* in F_1 after a cross or in delayed symptom plants in F_2 show this reaction.

Differential symptoms on peas have been recorded (Zaumeyer and Wade 1936; Smith 1937) as responses to inoculation with this virus and other related viruses, and have been attributed to plant age, environmental conditions, and variety. All of the above three symptoms types without timing have been described but have not been associated with a genetic scheme that was suggested in observations on this material.

IV. DISCUSSION

As pointed out by Holmes (1954) single genes have proved adequate to confer resistance to virus infection in many of the cases so far investi-

gated. In nearly all of these the dominant allele has been the one responsible, usually by rendering plants hypersensitive to infection. In Robust bean, however, immunity to typical strains of common bean mosaic is conferred by the homozygous condition of the recessive gene *aa* (Ali 1950).

In the experiments described above, immunity to pea mosaic virus occurs when plants are homozygous for the recessive gene *mo*. Plants of genotype *Mo Mo* are susceptible, while the presence of one of the recessive alleles confers a degree of tolerance to infection, typified by delay in symptom development and lack of stunting.

In breeding programmes, where populations to be inoculated may be necessarily small, selection of plants showing delayed symptoms could be useful in obtaining a high proportion of plants with the immunity gene in the heterozygous state.

Within the susceptible *Mo Mo* genotypes there are differences in reaction to infection with the strain of virus used for most of the testing. Plants of the Blue Prussian variety die after showing mosaic mottling, while Greenfeast plants, although developing mosaic, survive to produce seed. In this case of differential susceptibility, differences are not transmitted in crosses with consistency. In the cross Blue Prussian $\times$ William Massey a high proportion of the F_2 plants developing early symptoms later died. No information is available for the Blue Prussian $\times$ Onward cross which was inoculated with a different virus isolate. In Greenfeast crosses a high proportion of deaths in the early-symptom F_2 plants occurred with William Massey as immune parent but not with Onward. It thus appears that the variety William Massey in crosses with susceptible varieties, which are themselves different, can contribute to the development of systemic necrosis and subsequent death. The suggestion that this variety is "less immune" than Onward is contradicted by the small F_1 data where crosses in which it is involved tend to show delayed symptoms compared with the Onward crosses. This apparently complex virus-host relationship cannot be adequately explained by the available information.

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EXPLANATION OF PLATE 1

- Fig. 1.—Expression of pea mosaic symptoms on two genotypes. Left, mottling without necrosis on heterozygote Blue Prussian $\times$ William Massey. Right, necrosis following mottling on homozygous dominant Blue Prussian. Photo, J. W. Endt.
- Fig. 2.—Pea mosaic symptoms on Greenfeast. Infected plant (right) shows mottling and stunting. Healthy plant on left. Photo, J. W. Endt.

IMMUNITY TO PEA MOSAIC VIRUS



AN EXPERIMENTAL INVESTIGATION OF THE HEPATOTOXIC AND OTHER EFFECTS ON SHEEP OF CONSUMPTION OF *HELIOTROPUM EUROPAEUM* L.: HELIOTROPE POISONING OF SHEEP

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Summary

Experiments were conducted in which two flocks of breeding ewes were grazed on natural pastures containing *H. europaeum* L. A third flock was grazed on an area on which irrigation water was used to favour germination and early growth of the plant.

After the sheep had grazed the plant for the first period, a small proportion, from 1 to 7 per cent., died with damaged livers. After they had again grazed the plant during the following year, the death rate from liver disease rose to 50 per cent. in the exploratory experiment, to 38 per cent. in the first controlled flock experiment, and to 70 per cent. in the second controlled flock experiment.

Haematogenous jaundice was present in approximately half the sheep that died. Haemoglobinaemia with haemoglobinuria was present, usually in a mild form, in 31 per cent. of the deaths during the second year of the first controlled experiment, and in 5 per cent. during the second year of the second controlled experiment.

In the autumn and spring when the pastures were lush, many of the sheep while in good bodily condition died suddenly without being seen to be sick. Later, most of the deaths were in sheep that had lost condition.

Clinical pathological examinations showed that the affected sheep commonly exhibited a fall in haemoglobin and a rise in bilirubin in the blood. Tests showed a fall in prothrombin values. There were indications of a fall in plasma albumen, and also a fall in ability to store glucose.

Chemical analyses of livers collected from sheep that died showed the copper concentration to be above normal values, approximately 80 per cent. being over 1000 p.p.m.

Histopathological examinations of livers showed that the pathognomonic change was an increase in size of the liver cells associated with their increased death rate and loss of regenerative powers. The changes resulted in an atrophic hepatitis with little replacement fibrosis. There was an accumulation of ceroid in the livers, and a terminal occurrence of inclusion globules in the liver cells.

Related field investigations confirmed the findings of the experiments, and showed that the liver damage produced by the consumption of *H. europaeum* predisposed sheep to the haemolytic crisis of chronic copper poisoning.

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I. INTRODUCTION

The observations and results recorded in this paper were obtained during an investigation of a disease of sheep which had been called "yellows" by stockowners, and was later called toxaemic jaundice. The disease was known to occur in New South Wales and in northern Victoria. Edgar *et al.* (1941) have described its symptomatology, distribution, and incidence in New South Wales. It was first recognized "as a disease entity" in 1926 when it occurred in the Carcoar district, and then a little later in the adjoining Forbes district. From 1930 onwards it occurred frequently in the southern Riverina. Chamberlin (1933) had described the disease as being sudden in onset and usually fatal, and as being characterized by anaemia, intense icterus, and haemoglobinuria. In the later stages of an outbreak the disease ran a more protracted course. It was found to be enzootic in occurrence and to be confined to British breeds of sheep or to the first cross with the Merino. Usually the affected sheep were in good bodily condition, and the incidence in affected flocks varied from high to low. He carried out haematological investigations on the more acute type of cases occurring in an outbreak, but did not report results of the examination of the more protracted type. He found that destruction of red blood cells occurred rapidly, and that about three-quarters of the circulating cells were destroyed. Punctate basophilia and nucleated red cells occurred along with a leucocytosis. Albiston (1929) described an outbreak of a similar disease which occurred near Seymour, Vic. He was unable to find an infective cause, and suggested that it might possibly be due to the consumption of a poisonous plant with a delayed action, although he found no evidence to support the suggestion.

Rose and Edgar (1936) investigated haemolytic jaundice in sheep and cattle, and concluded that the disease in both species is an enterotoxaemia due to *Cl. welchii*, type A. They mentioned that Seddon (unpublished data) had exhaustively tested the theory of causation of toxaemic jaundice of sheep by a toxic plant or plants, without obtaining evidence to support it.

In 1938 a cooperative investigation of the disease was arranged. The hypothesis that the haemolytic jaundice which had been named toxaemic jaundice of sheep was a manifestation of chronic copper poisoning was taken as the starting point. The plan was to carry out field investigations of outbreaks in commercial flocks, and to study the disease systematically under field-station conditions in an area where it was enzootic and had a high incidence. We (Albiston *et al.* 1940) recorded the results of the early field investigation carried out in north-eastern Victoria and eastern Riverina. The results proved beyond doubt that these particular outbreaks of disease, characterized by haemoglobinuria and haemolytic jaundice, were due to chronic copper poisoning. A preclinical rise of blood copper, which was steep and about tenfold in value, was found in sheep which developed the disease. This was the first demonstration of the occurrence

of chronic copper poisoning in sheep grazing improved or native pastures in the absence of any extraneous contamination with copper salts or minerals. A problem of some complexity was thus revealed.

In 1939 a systematic study of the disease was begun on a property in southern Riverina where the disease had been enzootic, with a high incidence, for several years. The experimental flocks of sheep, mainly first cross of Merino with one of the British breeds, were used for fat-lamb production. The animals were thus subjected to the usual stresses of a breeding flock, and were grazed for the most part on the native unimproved pastures common to the region as described by Moore (1953).

The exploratory experiments were started in January 1940 and completed by the end of 1943, when it had become apparent that the action of *Heliotropium europaeum* L. on the health of sheep must be made the subject of special study, although earlier work at the Veterinary Research Station, Glenfield, N.S.W., had failed to give any indication that it possessed harmful properties.

During the progress of the investigation, reference was made in the annual reports of the Investigation Committee (Anon. 1948-1950) to the discovery of the harmful effects of consumption of *H. europaeum* by sheep.

In the following sections of this paper are presented the design of the experiments on the toxic effects of *H. europaeum* and an outline of the results obtained.

II. FLOCK EXPERIMENTS

(a) *Exploratory Flock Experiments (Experiment A)*

The field station, of 400 acres, was situated in the southern Riverina district, about 4 miles from the township of Barooga. It is in the Riverine Plain of south-eastern Australia, which has been described by Butler (1950).

A flock of 306 Border Leicester $\times$ Merino ewe hoggets was placed on the property, and brought under observation in January 1940. The experiment was originally designed to investigate the possibility of keeping crossbred ewes free from toxæmic jaundice by limiting the amount of copper stored in the liver. With this in view, one-half of the flock had access to a salt lick containing ferrous sulphide, which had been shown by Dick (1954) to limit copper storage by sheep. The two groups were rotated between two paddocks at fortnightly intervals, the sulphide lick being moved with the one group. The ewes were mated in January to Southdown rams. This regime with the sulphide lick was continued until April 1942; its use was then stopped, and the groups were rearranged to test the effect of nutritional level by providing a restricted grazing for one and unrestricted for the other. This second phase continued until July 1943. In the third phase, one group was provided with a supplementary ration of chaffed oaten hay and lucerne hay; this continued until November 1943.

During the first 26 months, 11 sheep in the sulphide group and 6 sheep in the control group died. None appeared to die from toxæmic jaundice. Some deaths were associated with lambing. During the summer and early autumn of 1940 there were no effective falls of rain; the pasture consisted mainly of dried grasses and native clovers with patches of *Heliotropium europæum* in the green, flowering stage. This summer-growing plant persisted until killed by the first frosts in May. In April, 3.75 in. of rain fell and an abundant growth of *Erodium cygnorum* Nees and *Hordeum leporinum* Link occurred. The pasture became grass-dominant, with annual grasses constituting about 70 per cent. and perennial grasses about 10 per cent. By November the annual grasses had matured, seeded, and become dry, but the perennial grasses showed more active growth. A few seedlings of heliotrope were seen in some places favoured by moisture. A fall of 4 in. of rain in January 1941 destroyed the food value of the dry grass, but caused heliotrope to germinate and become widespread in the pasture. In March, 4.5 in. of rain fell, giving renewed growth of heliotrope which remained green and dominant (about 90 per cent.) in the pasture until the end of May. The rainfall in the summer of 1941-42 did not favour the germination and growth of heliotrope. The sheep remained healthy and in good condition. The death rate from all causes during this period of 26 months was 5.7 per cent.

A death occurred in a ewe in fat condition in June 1941, 17 months after the commencement of the experiment. It belonged to the control group with no access to ferrous sulphide. Its liver was small, 290 g, and contained 2250 p.p.m. copper. There was no evidence of haemoglobinuria, but the presence of a high concentration of copper suggested disturbance of the copper metabolism. (In comparison, the liver-copper concentration of some sheep that died in the sulphide group during 1940 and 1941 varied from 34 to 790 p.p.m.) The high concentration of liver copper did not appear to be the cause of death; however, in the light of present knowledge it is probable that this animal died from liver damage due to the consumption of *H. europæum*. Up to this time it had been impracticable to obtain sections of liver and kidney for histopathological examination.

At the end of this period of 26 months, a sheep in the sulphide group died in January 1942 with a relatively small liver having a copper concentration of 2550 p.p.m. Two weeks later another sheep in the control group died; it showed a small damaged liver with a copper concentration of 1420 p.p.m. Therefore, of the 17 sheep that died during this first period, at least 3 probably died as the result of liver damage from the consumption of *H. europæum*, although at the time the association of the plant with morbid changes in the sheep was not suspected.

Because the ferrous sulphide lick had failed to give any clear-cut results, and because the high nutritional level of the sheep in both groups

may have prevented an outbreak of the disease, the sheep were equally divided into two new groups, one to be given restricted and the other unrestricted grazing. The restricted-grazing group grazed a pasture comparable to that of the other group, but the amount of food available was restricted for the most part by heavy stocking with additional non-experimental sheep. During this period, which continued for 15 months, the rainfall in May and June was good, and brought about a heavy growth of pasture during the winter and spring months. In November, 3.5 in. of rain fell. It caused germination of heliotrope which grew well throughout the summer and autumn, and lasted until about the end of May 1943. This second phase was brought to a close in June 1943. During the 15 months, 98 sheep died, 68 in the restricted-grazing group and 30 in the unrestricted. Of these 98 sheep, 57 had been earlier in the sulphide group and 41 in the control group. Thus the earlier and later effects of the ferrous sulphide intake by the sheep may have been harmful. It seemed from the results in this second period that a higher food intake may have had some protective value, although there was not a very great difference in the body weight of the sheep in the two groups, the one being only about 10 per cent. lower than the other. Nevertheless, the losses started earlier, and the death rate in the restricted group was more than twice that in the other.

The third phase of the experiment, from July to December 1943, was carried out to determine the value of a supplementary diet. The sheep were divided equally to constitute two new groups, one of which was given chaffed oaten hay in self-feeders, and lucerne hay. The rainfall during the previous 6 months had been low. Light showers in May and June had started the winter-growing plants, but lack of soil moisture and heavy frosts had inhibited further growth of the pasture. Good pasture growth took place in August, and the supplementary feeding was stopped at the end of the month. Some germination of heliotrope occurred in October but the strong growth of grass and lack of rain in November and December prevented the plants from developing. During this period of 6 months, 50 sheep died, 33 in the group which had received supplementary feed, and 17 in the control group. This result failed to support the suggestive result obtained in the second period when a higher death rate occurred in the group under restricted grazing conditions.

Taking 1943 from January to the end of December, the death rate in the flock was 50 per cent., whereas during 1941 and 1942 it had been 1.7 and 5.2 per cent. respectively. The death rate first began to increase in November 1942. It then declined somewhat for 3 months, and started to rise steadily during March and April. In May and June a peak was reached, followed by a fall. In November and December it rose again. These vital statistics are shown in Figure 1.

Thus an outbreak of disease was obtained in this experimental flock. Its character differed from that of the outbreaks of chronic copper poisoning which we had investigated earlier (Albiston *et al.* 1940). Although

Year	No. of Sheep													Annual	
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Death Rate (%)
1940	300													10	3.3
1941	290	1	—	—	—	—	2	1	—	—	—	—	1	5	1.7
1942	285	1	1	—	—	—	—	1	1	—	—	9	2	15	5.2
1943	269	1	3	6	7	30	35	16	3	—	7	11	16	135	50.0

— For period of first phase (ferrous sulphide lick)

== For period of second phase (restricted and unrestricted grazing)

=== For period of third phase (supplementary feeding)

Fig. 1.—Experiment A: secular occurrence of deaths in all groups combined.

clinical and post-mortem signs of jaundice were present in many of the sheep that died, they were absent in others. Some developed lesions of photosensitization about the face and ears in September 1943. Haemoglobinuria was observed in 2 animals only of the 165 which died from other than accidental causes. Systematic histopathological examinations were not carried out until May 1943 when the steep rise in the death rate occurred. Because most of the deaths were more or less sudden, some carcasses were not found until post-mortem decomposition had destroyed their value. Nevertheless in a good proportion, specimens of liver and kidney were collected for microscopic examination. In 14 of the 165 sheep that died during the 47 months, evidence was found in sections of the kidney of the passage of haemoglobin into the nephron: a few haemoglobin casts were present in the tubules and granules in the epithelial cells giving a positive Prussian blue (P.B.) reaction. In 2 only of the 14 cases were the casts numerous, but in neither was haemoglobinuria observed during life; in 3 the evidence was restricted to the presence in the epithelial cells of the tubules of granules which gave a positive P.B. reaction; in 4, evidence of jaundice was not observed during life or at autopsy.

It was clear that we were not dealing with the usual manifestations of the haemolytic crisis of chronic copper poisoning in sheep. Histopathological examination of liver from the dead animals showed very definite liver damage with megalocytosis of the parenchyma cells, and usually slight fibrosis. Later we found that these changes were characteristic of liver damage following consumption of *H. europaeum*. At one stage, the livers were very fatty; usually about 20 per cent. of the wet weight, but reaching, in several instances, over 40 per cent. The livers usually contained a high copper concentration, over 1000 p.p.m. and up to 3650 p.p.m. as a maximum. When the fat content of the liver was above normal it was necessary to estimate the copper concentration on a fat-free dry weight basis. The high concentration of copper in the liver was an outstanding phenomenon. Although the characteristic haemolytic crisis of chronic copper poisoning in sheep was not present, we had to consider the possibility of a chronic copper toxicosis as the cause of the liver damage. Systematic analysis of the pasture plants for copper had failed to reveal values above recognized normal levels (Dick, Moore, and Bingley 1953). The presence of *H. europaeum* in the pasture during the summer months appeared to have definite significance in relation to the occurrence of the disease. The plant had a copper content ranging between 10 and 15 p.p.m., slightly higher than most of the other plants in the pasture. For this reason, and also because *H. europaeum* cannot be regarded as a normal pasture plant, it was decided that the part it may play in the production of the disease should be investigated by appropriate experiments.

(b) *First Controlled Flock Experiment (Experiment B)*

(i) *First Year*.—The plan was to purchase a new flock of young ewes from a district where toxæmic jaundice did not occur. The flock was to be divided into two groups of equal numbers, one to have access to and the other to be excluded from *H. europæum*. Because field experience suggested that the disease occurred more readily in sheep on a lower plane of nutrition, each group was equally subdivided to provide one-half (H) with supplementary food and the other (L) with restricted intake. So far as was practicable the sheep were to be grazed on the natural pasture on the field station but, since the rainfall was unreliable and adequate growth of *H. europæum* uncertain, an irrigable area adjacent to the field station was obtained.

In November 1943, 301 Border Leicester $\times$ Merino ewe hoggets were brought to the station. One area was heavily grazed by introduced sheep to reduce the amount of feed available to subgroups (L), to be kept on a lower plane of nutrition. The area to supply heliotrope for one of the groups received its first watering early in December. It was watered again on January 21, 1944. The rainfall during 1943 had been low, the total being about 10 in., of which 1.5 in. fell in September, 1.0 in. in October, 0.5 in. in November, and none in December. At the end of the year the pasture was completely dry; there had been no growth of heliotrope. We were able therefore, without special arrangements, to keep the non-heliotrope group free from any exposure to the plant. The (H) subgroups to be maintained on a higher plane of nutrition were placed on the best pasture available, and were supplied with a supplement of lucerne hay and crushed wheat at the end of December. Towards the end of January 1944 the mean body weight of the (L) subgroups was 81 lb and that of the (H) subgroups 86 lb.

On February 19, 1944, the appropriate group of 148 sheep, half having been on a restricted diet, was placed on the irrigated block to graze the heliotrope. The sheep grazed the plant well; there were few other pasture plants available. Except for 30 ewes which remained for a further 18 days, they were removed from this irrigated pasture on April 11, and placed to graze on the natural pasture at the station where heliotrope had not grown because of the lack of rain during January and February. Thus the period during which this group of sheep grazed heliotrope almost exclusively was 52 days. Under natural conditions heliotrope is usually available for a somewhat longer period than this. However, the amount of the plant consumed was much greater than sheep could consume under natural conditions in a mixed pasture. Although the plant is not very palatable to sheep, they appear to overcome the early dislike to it, especially when it is the only green plant in a pasture.

The sheep in the (L) subgroup increased in mean body weight from 81 lb when placed on the heliotrope pasture to a mean of 93 lb when

removed. Likewise, those in the (H) subgroup increased in mean body weight from 86 to 97 lb. They had been mated, and most of them were pregnant. The evidence is that the green heliotrope proved an adequate maintenance diet.

When the sheep returned to the field station, those in the (H) subgroup were again given a supplementary diet. The rainfall in April was 1.5 in., which was followed by 2.5 in. in May, after which a fresh growth of pasture occurred. Little of the supplementary ration was eaten after this for some time but, as the spring rainfall was low and the pasture growth was poor, supplementary feeding with lucerne hay was recommenced in September. About an inch of rain fell in October and another inch in November, after which a few seedlings of *H. europaeum* appeared in the pasture. High winds and dust storms caused the disappearance of most of these seedlings. However, precautions had to be taken to keep the control group of sheep on portions of the property where heliotrope did not occur.

In October, 10 sheep from the control and 10 sheep from the heliotrope groups were sent to slaughter to provide livers for pathological and chemical examinations.

Results.—For the period of 12 months from the time the group of 148 ewes was placed on the irrigated block to graze on the heliotrope until the end of February 1945, there were 16 deaths in this group, 14 in the (L) and 2 in the (H) subgroups. This represents a death rate of 11.6 per cent. for the period. The first death occurred on May 17, about a month after the grazing on the heliotrope had ceased. The number of deaths occurring in this heliotrope group each month from May 1944 to February 1945 is shown as follows:

1944								1945			Total
May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.		
1	—	1	1	3	5	—	3	2	—		16

The first sheep to die in May was seen to be ailing for 4 days. Its liver was very small, 180 g, and contained 2900 p.p.m. copper on the dry weight fat-free basis. From the evidence obtained from pathological examination it is judged to have died as a result of liver damage from the consumption of heliotrope. Some of the sheep developed lesions on the face due to a photosensitization. In some of the sheep these lesions became infected, which led to abscess formation. This photosensitization is judged to have been due to a degree of liver damage which rendered the organ unable to deal efficiently with the removal of phylloerythrin from the circulation. Death of these animals may therefore be regarded as due to an intercurrent infection to which the animals were predisposed by the liver damage produced by the consumption of heliotrope. The cause of death was not determined in all these 16 animals. Five of the carcasses were not recovered sufficiently early to enable pathological examinations to be made. One of

the animals which died in December showed no evidence to suggest that consumption of heliotrope was responsible. Thus of the 16 deaths, 6 were probably directly due to advanced liver disease, heliotrope hepatitis, and 4 to intercurrent infection of facial lesions of photosensitization, and in 6 the cause of death was not determined.

In the control group, which could have consumed little or no heliotrope, 5 sheep died during the 12-month period, all from the (L) subgroup. One of these died from post-partum haemorrhage, and the others from the effects of the restricted food intake. The death rate for the period was 3.6 per cent.

The heliotrope grazing by the sheep, for a period of 52 days for most of the group and of 70 days for 30 of them, had failed to produce an outbreak of the disease under investigation, although there was some evidence that liver damage had directly caused the death of 5 with the development of haemoglobinuria and jaundice in 2 of them. It was therefore decided that the experiment should be continued to include a second exposure to the effects of the consumption of *H. europaeum*.

(ii) *Second Year*.—The irrigated block was watered between December 6 and 12, 1944, and again towards the end of January 1945. The experimental group of sheep, which now consisted of 122 animals, was placed on February 3 to graze on the heliotrope, which had responded well to irrigation. In December 1944 the rainfall was 1.77 in. and in January it was 3.48 in. This rainfall caused germination and growth of heliotrope on some portions of the field station. It therefore became necessary to remove the sheep in the control group to pastures on a neighbouring property where heliotrope had not been known to occur. The sheep thus removed were those in the (H) subgroup. The (L) subgroup was kept on a well-grazed portion of the field station where heliotrope did not appear.

There was no effective fall of rain during February, March, April, and May. Therefore the sheep on the field station had to be maintained on conserved forage, the (H) subgroup receiving an additional supplement of wheat.

The sheep had eaten out the heliotrope on the irrigated block by the end of March. They were therefore returned to a portion of the field station which had been rested from grazing, and where heliotrope dominated the pasture. The heliotrope in this situation was eaten out by April 19; the period of heliotrope grazing was thus completed. The period was 44 days, which is somewhat shorter than that in the first 12-month period.

In the absence of autumn rains there was no regeneration of the pastures until June 1945, when 2.19 in. of rain fell. The rainfall in the following months was as follows: July, 1.35 in.; August, 2.46 in.; September, 0.32 in.; October, 2.12 in.; November, 1.55 in.; December, 0.29 in. The

pasture grew well in the spring. A few patches of heliotrope were observed in December. In January 1946 the rainfall was 1.6 in., and in February 4.70 in., when an abundant growth of *Erodium cygnorum* and *Hordeum leporinum* occurred.

Results.—During the period of 12 months ending February 1946, there were 50 deaths among the group of 122 sheep which had grazed the heliotrope for the second consecutive year, a death rate of 41.0 per cent. Of these 50 deaths, 28 occurred in the (L) subgroup and 22 in the (H) subgroup. The distribution in time of deaths in the two subgroups was approximately equal. On each of the two occasions when the sheep had been placed to graze on the heliotrope both subgroups had improved in body weight. Thus, judged by body weight, the (L) subgroup of the heliotrope group had an advantage over the (L) subgroup of the non-heliotrope or control group.

The monthly incidence of deaths in the heliotrope group is shown as follows:

1945						1946			
Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. Feb. Total
3	15	11	2	2	—	2	2	6	7 — 50

It is seen that the peak was reached in May-June, and that another smaller rise occurred in December-January. Of the 50 animals that died, 10 had been found too long after death to enable adequate autopsy and pathological examinations to be made. It is probable that the majority of these died with advanced liver damage. In the others, clinical and pathological examinations revealed the cause of death with some degree of certainty. Death of 2 of the 50 animals was associated with intercurrent infection, but a moderate degree of liver damage was present. Jaundice was present, on the basis of clinical or pathological examination, or both, in 22 (52 per cent.) of 42 animals adequately examined. Haemoglobinuria was present on clinical or pathological examination, or both, in 13 (31 per cent.) of the 42 animals examined. In 3 of the animals that died, hepatotoxic effects of heliotrope could be excluded as making any definite contribution to the lethal results.

During the same period of 12 months, there were 14 deaths in the 133 sheep in the control (non-heliotrope) group. Of these, 11 were in the (L) subgroup, and 3 in the (H) subgroup. The death rate was therefore 10.5 per cent., which is high for a normal breeding flock of ewes. The proportion of deaths in the (L) subgroup is significantly higher than in the (H) subgroup, and thus differs from what occurred in the heliotrope group. This (L) subgroup of the control group was under greater nutritional stress than the comparable subgroup in the heliotrope group. One of the 14 animals, which belonged to the (H) subgroup, developed clinical jaundice and died in June 1945 with haemoglobinuria. The liver cells showed megalocytosis, and the nature of the damage made it certain

the animal had had access to heliotrope, although advanced liver damage was not the immediate cause of death. Death in 4 others was associated with an intercurrent infection, in 3 with disorders of pregnancy, in 4 with poverty and emaciation, and in 2 the cause of death could not be determined as the carcasses had not been recovered early enough. The microscopic examination of the liver showed definite damage in the one animal that died with haemoglobinuria. In 5 other animals there were present some large phagocytes containing ceroid. These have been found to be frequent in the liver of sheep that have had access to heliotrope. Therefore, although none of these sheep died from the advanced liver damage seen in the dead sheep of the heliotrope group, there was this evidence that the sheep in the control group had consumed a small amount of heliotrope.

The results of the chemical examination of the livers of sheep from both groups must be considered possibly as offering further evidence on the effects of the consumption of heliotrope. If a rise in liver-copper concentration occurs in sheep as a consequence of the consumption of heliotrope, a similar rise in the sheep in the control group may be taken as supporting evidence that the group consumed some of the plant.

In Table 1 figures are given of the copper concentration in the liver of sheep in the two groups which died or were slaughtered. The concentration was determined on a fat-free dry weight basis. In the animals that were slaughtered, the fat content of the liver was not determined but the figures have been adjusted for purposes of comparison, on the assumption that the mean moisture content was 70 per cent. and the mean fat 5 per cent., a rational assumption for relatively healthy sheep. The mean figures are divided into two groups, viz. a group in which the liver-copper concentration was 1000 p.p.m. or over and a group in which it was under this level. This grouping is adopted largely because of our experience with chronic copper poisoning in sheep in which we have found the concentration of 1000 p.p.m. to be critical: sheep rarely die from the haemolytic crisis unless the concentration has reached or exceeded this level.

The figures show that, of the animals dying in the heliotrope group during the first 12 months, 45.4 per cent. had acquired a liver-copper concentration of over 1000 p.p.m. During the second period of 12 months the proportion rose to 83.3 per cent. In the control group, one animal of 5 that died in this first period of 12 months showed a concentration over 1000 p.p.m. However, in the second period of 12 months, the proportion rose to 53.8 per cent. in the control group as against 83.3 per cent. in the heliotrope group.

This problem of the possible action of heliotrope in raising the copper concentration in the liver of sheep is discussed further in the later sections. At this stage it may be said that the rising liver-copper status of the sheep

TABLE 1
COPPER CONCENTRATION IN THE LIVER OF SHEEP DYING OR SLAUGHTERED IN EXPERIMENT B
Concentration on fat-free dry weight

Period (died or slaughtered)	Group	Total No. Livers Examined	Over 1000 p.p.m. Cu			Under 1000 p.p.m. Cu		
			No.	%	Mean (p.p.m. Cu)	No.	%	Mean (p.p.m. Cu)
First 12 months (died)	Heliotrope Control	11	5	45.4	2035	6	54.5	603
		5	1	20.0	(1820)	4	80.0	774
At 9 months (slaughtered)	Heliotrope Control	8	2	25.0	1510	6	75.0	443
		8	0	—	—	8	100.0	548
Second 12 months (died)	Heliotrope Control	36	30	83.3	1566	6	16.6	738
		13	7	53.8	1180	6	46.1	636
Post-experimental (slaughtered)	Heliotrope Control	64	10	15.6	1150	54	84.4	549
		117	15	12.8	1426	102	87.2	548
Combined totals	Heliotrope Control	119	47	40.0	—	72	60.0	—
		143	23	16.0	—	120	84.0	—

in the control group may be taken to support the more specific histopathological evidence that the sheep consumed some heliotrope during the 24 months of the experiment, although not enough to bring about death from frank liver damage. There is further support for this in that three of the remaining animals died a little later, one in April, one in September, and one in October. The last animal was seen to be ailing for 5 days, developed haemoglobinuria and jaundice, and was killed *in extremis*. Microscopic examination showed megalocytosis of the liver cells, which leaves no doubt that it had consumed some heliotrope.

The sheep remaining alive after this were sent to slaughter during October, November, and December 1946. Livers were collected and subjected to chemical and histopathological examination. The results of the chemical examination for copper are given in Table 1. It is seen that 15.6 per cent. of the residue sheep in the heliotrope group had livers containing over 1000 p.p.m. copper as against 12.8 per cent. of those in the control group. Thus with the passage of time, the control group showed a trend towards catching up with the heliotrope group. However, over the whole period of 24 months, 40 per cent. of 119 livers examined from the heliotrope group had copper concentrations of over 1000 p.p.m., whereas only 16 per cent. of 143 livers in the control group reached this level.

The results of the experiment left no doubt that *H. europaeum* was a poisonous plant with hepatotoxic properties, and that a proportion of the sheep dying as a result of its consumption might develop clinical or pathological evidence of haemoglobinuria. The haemoglobinuria was sometimes frank and severe, but more often it was relatively slight and subclinical. The relationship between this destruction of red blood cells and the high copper status acquired by many of the sheep was not revealed by the experiment. It was clear that the consumption of *H. europaeum* could cause severe liver damage which might result in death from loss of function of the organ, and that haemoglobinaemia was not an essential part of the lethal effect. However, the development of haemoglobinuria in two sheep in the control group, which also showed megalocytosis without appreciable destruction of the liver cells, strongly suggested that the consumption of *H. europaeum* in relatively small amounts over two consecutive years may predispose to the development of the haemolytic crisis of chronic copper poisoning.

It became necessary, therefore, to carry out a further experiment in which the control group was successfully prevented from consuming any of the plant, *H. europaeum*.

(c) *Second Controlled Flock Experiment (Experiment C)*

(i) *First Year.*—The plan for the second controlled experiment was to allow one half of a new flock of crossbred ewes to have free access to *H. europaeum*, that might be expected to grow in the natural pasture of the field station, but to protect the other half from any access to the plant.

The growth of *H. europaeum* would depend on the occurrence of rain during the warm months of the year. The plan would fail if suitable rainfall did not occur. To protect the control group from access to *H. europaeum*, cereal crops and hand-feeding would have to be used.

A new flock of 306 weaner ewes was obtained in October 1946. Ten were slaughtered for the determination of pre-experimental liver-copper values. The sheep were weighed and divided at random into two groups at the end of October. The mean body weights of the two groups were found to be even.

Rainfall had been almost absent in September, but in October it was 0.80 in., in November 2.84 in., and in December 1.14 in. The pasture was dry, but the feed consisted largely of dry *Erodium cygnorum* and was ample for the sheep in October. The control group was placed, at the end of October, on an area sown to oats and Wimmera ryegrass. On December 10 it was found that the mean body weight of the control group had increased by 21.5 lb, whereas that of the natural-pasture group had increased by 3.4 lb. By this time there was a good germination of heliotrope in the natural pasture. Thus the pre-experimental arrangements were completed during November and December, and the early summer rainfall had brought about the germination of heliotrope, which was available for the natural-pasture (heliotrope) group.

The first year of the experiment is, therefore, regarded as extending over the calendar year 1947. During this period heliotrope was available to, and was eaten by, the natural-pasture group from January to May inclusive. It grew abundantly in the main grazing areas of the field station. In February, C. W. E. Moore found that, on a weight estimate, heliotrope constituted from 88 to 97 per cent. of the pasture material available to the sheep. The sheep during these months subsisted largely on it although they had a mixed diet, since *Stipa* spp. and *Danthonia* spp. were also available. During the winter and spring months the pasture was dominated by *Erodium cygnorum* and *Hordeum leporinum*. *H. europaeum* became available again as seedlings in November and December. During December, two of the sheep in this group strayed and were lost to the experiment.

The management of the control group was more difficult. The sheep were placed at the end of October 1946, on a cultivated area of the field station where oats and Wimmera ryegrass had been sown, but they had to be given a supplement of chaffed oaten hay towards the end of January 1947. At the end of February they were removed from this area because some heliotrope had appeared in the pasture. Some of the sheep, no doubt, consumed a small amount of the plant. They were placed in a yard and completely hand-fed on chaffed oaten hay until the end of June. Under these conditions they lost a little weight, but were still about equal to the natural-pasture group in bodily condition. They were returned to the cultivated area in July when all danger of their finding any heliotrope to

eat had disappeared. They remained there for the rest of the year, and increased in body weight.

Results.—The natural-pasture (heliotrope) group consisted of 146 sheep, but before the end of the year 2 had strayed; the control group consisted of 148 sheep. During the year the death rate in the natural-pasture group was 7.6 per cent., i.e. 11 sheep died; in the control group it was 3.5 per cent., i.e. five sheep died.

Of the 11 deaths in the natural-pasture group, 2 occurred in July, 3 in October, and 6 in November. The carcass of one of these animals was not recovered soon enough to enable the collection of satisfactory specimens for pathological examination, but the autopsy failed to reveal the presence of bile-staining of the tissues. One animal that died in July showed clinical haemoglobinuria and well-developed jaundice. One that died in November showed moderate jaundice but no haemoglobinuria. One died at lambing, but specimens could not be collected for examination. Clinical, autopsy, and pathological examinations showed that 9 of the 10 animals had liver damage sufficiently advanced to have caused death, or to have contributed to its cause. Lesions of photosensitization, which occurred in a proportion of the sheep in this group, appear to have contributed to the cause of death in 2 of the animals.

Of the 5 deaths in the control group, 3 occurred in July and 2 in October. None of the sheep was pregnant. One probably died from enterotoxaemia. One had developed an ophthalmia, had become cast, and was killed *in extremis*. One carcass was too decomposed for examination. In the 4 animals providing tissues for pathological examination there was no evidence of any damage in the liver or in the kidney.

(ii) *Second Year.*—The rainfall in November and December of the previous year was 1.10 and 3.62 in. respectively. This assured the continued growth of *H. europaeum* in the natural pasture, but it was not quite so abundant as in the previous year. There was no rain in January, but in February 2.97 in. fell.

Because heliotrope appeared in the cultivated area, the control group was placed in the yards on February 14 and was hand-fed on chaffed oaten hay until the middle of May, when it was taken back to the cultivated area. In June it was transferred to the natural pasture; it remained until the middle of August when it was again taken back to the cultivated area. It had another short period on the natural pasture in November, but had to be returned to the cultivated area in December because heliotrope had appeared again in several areas of the natural pasture. Thus, during the first and second years of the experiment the sheep in the control group could have consumed only a very small amount of heliotrope, but it is certain that some was consumed at least by a proportion of the sheep.

The natural-pasture (heliotrope) group consumed the heliotrope which grew fairly abundantly in the pasture until the end of April. By

the middle of May the heliotrope had been completely destroyed by the frosts. Although there was no rain in March, in the 3 following months sufficient fell to enable the perennial grasses and the annual herbage to grow, and to provide reasonably good grazing for the sheep. The rainfall in July and August was low, but the pasture remained in a satisfactory condition despite the dry winter. The mean body weight of the natural-pasture group rose slightly from 89.9 lb in April to 96.6 lb in October, but the mean for the control group at these times was 108.8 and 123.2 lb respectively. There were only 56 sheep remaining in the natural-pasture group to be weighed on October 19. Their body weights ranged from 53 to 146 lb; 10 of the 56 had body weights above the mean of the control group. There were very few light-weight sheep in the control group at this time; of 136 sheep, 6 had a body weight between 90 and 100 lb, the range being from 91 to 168 lb. Although the control group had been given favoured pasture conditions when they were placed on the cultivated area, they invariably lost weight when being completely hand-fed in the yards. The low mean value for body weight of the natural-pasture (heliotrope) group in the later stages of the experiment was therefore mainly due to loss of health.

Results.—During this second year of the experiment ending December 31, 1948, there were 93 deaths among the group of 133 sheep in the natural-pasture (heliotrope) group, a death rate of 70 per cent. The monthly incidence of deaths in this group is shown as follows:

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
2	1	2	2	—	7	10	24	18	8	15	4	93

The peak of the outbreak occurred in August, followed by a fall in September and October, and a slight rise in November.

Of the 93 animals that died, 22 had been found too long after death to enable adequate autopsy and pathological examinations to be made. In a number of other instances post-mortem changes were advanced, but portions of liver and kidney were removed for microscopic examination. Most of these specimens of the liver enabled a limited examination to be made for bile pigment and for degree of liver damage. Thus, jaundice or bile-staining of the tissues was present on the basis of clinical or pathological examination, or both, in 27 (41 per cent.) of 66 animals adequately examined. Haemoglobinuria was present on clinical or pathological examination, or both, in 3 (5 per cent.) of 60 animals adequately examined.

Of the 7 sheep which died in the first 4 months of 1948, only 2 came to adequate pathological examination. Both showed heavy jaundice and haemoglobin casts in the kidneys, but neither had been seen to exhibit haemoglobinuria. Two others showed heavy bile-staining of the tissues at autopsy, but post-mortem decomposition had rendered the tissues unsuitable

for microscopic examination. The jaundice was almost certainly haematogenous. Thus 4 of the 7 animals probably had a haemoglobinuria of sufficient severity to contribute to the cause of death, but the livers showed damage sufficiently advanced to be the main cause of death.

Microscopic examination of the livers from the other animals which died during the year left no doubt that liver damage was the cause of death whatever else may have made any contribution to the fatal termination. The 22 carcasses which were not adequately examined were distributed at random; it is probable that liver damage was the cause of death in all cases, just as was found in the larger proportion which was adequately examined. The nature of the characteristic changes in the liver and kidney is discussed in a subsequent section.

During the same period of 12 months, there were 7 deaths in the 143 sheep in the control group, a death rate of 4.8 per cent. One died in April, 2 in May, 1 in July, 2 in August, and 1 in September. At least 3 of these deaths were associated with pregnancy or lambing troubles, one appeared to be due to enterotoxaemia, and one to a tumour near the heart. Jaundice or haemoglobinuria did not appear in any of the sheep, nor was any liver damage found in any of the animals that died. Deaths in this group did not occur after September. In view of this, and because the experiment was drawing to a close, 30 of the sheep were selected at random and slaughtered early in December. The livers from these animals were collected for chemical and microscopic examination.

Chemical analysis showed that 2 of the 30 sheep from the control group had acquired a copper concentration of over 1000 p.p.m. when slaughtered in December 1948. The results of the analyses of the livers of the sheep that died or were slaughtered in the experiment are given in Table 2. It is seen that in experiment C the proportion of sheep with livers containing 1000 p.p.m. or more of copper in the control group is 4.8 per cent. compared with 16.0 per cent. in the comparable group in experiment B. Microscopic examination of the livers failed to reveal the presence of megalocytosis of the liver cells or other form of liver damage. These results can be taken as clear evidence that the control group was kept from consuming heliotrope in more than minimal quantities.

(d) *Analytical Summary of Flock Experiments*

Observations on *H. europaeum* in the particular environment showed that the plant grows in the warmer months of the year when the rainfall is adequate. If about an inch of rain falls in November, germination of the seeds occurs; soil temperature is rarely high enough for this to occur earlier. The plant flowers very early after germination, frequently as early as the ninth day, before the cotyledons have faded. It grows fairly rapidly if temperature and moisture are favourable, and continues to branch and flower until destroyed by low temperature or frosts. Thus the

TABLE 2
COPPER CONCENTRATION IN THE LIVER OF SHEEP DYING OR SLAUGHTERED IN EXPERIMENT C
Concentration on fat-free dry weight

Period (died or slaughtered)	Group	Total No. Livers Examined	Over 1000 p.p.m. Cu			Under 1000 p.p.m. Cu		
			No.	%	Mean (p.p.m. Cu)	No.	%	Mean (p.p.m. Cu)
Pre-experimental	—	10	—	—	—	10	100.0	219*
First 12 months (died)	Heliotrope	7	3	42.8	2080	4	57.2	517†
	Control	4	—	—	—	4	100.0	378*
Second 12 months (died)	Heliotrope	54	42	77.7	1683	12	22.2	723‡
	Control	7	—	—	—	7	100.0	476‡
Post-experimental	Control	30	2	6.6	1198	28	93.3	457§
Combined totals	Heliotrope	61	45	73.7	—	16	26.3	—
	Control	41	2	4.8	—	39	95.2	—

* Individual values corrected for 5 per cent. fat.

† Corrected for 15 per cent. fat.

‡ Corrected on chemical analysis for fat.

§ 12/30 corrected on chemical analysis; 18/30 corrected for 5 per cent. fat.

plant is usually well grown by the end of December when the native and other plants in the natural pasture have matured and dried off. Under these conditions it becomes relatively attractive to sheep, mainly to the British breeds or to their first-cross with the Merino.

Seasonal conditions and the natural habits of the plant have therefore determined the period over which it is most appropriate to conduct a grazing experiment, viz. from January to December.

The controlled experiments proved beyond any doubt that *H. europaeum* has hepatotoxic properties. Pathological studies showed that a characteristic liver damage was produced in sheep. Clinical studies showed that jaundice was frequently but not invariably present in the diseased sheep. In examining the experimental results for statistical and epidemiological studies, we have depended on information from both clinical and pathological examinations for confirmation of a diagnosis, or for determination of the cause of death. It was found that death may result occasionally from loss of kidney function following a severe haemoglobinaemia, or more frequently from the direct loss of hepatic function. In the following epidemiological considerations, no attempt is made to draw any distinction between these two major manifestations since both are due to the same cause in these experiments.

(i) *Experiment A*.—The results of experiments B and C make it clear that the high mortality rate reached in the first flock used for experiment A was due to disease produced by the consumption of *H. europaeum*. During 1940 the plant appeared in the pastures in a few isolated patches only, therefore none of the 10 sheep that died (*vide* Fig. 1) showed evidence of hepatic disease. In the summer of 1940-41, the rainfall favoured widespread and plentiful occurrence of heliotrope in the pasture. An animal died in January 1941 but showed no evidence of hepatic disease. Two deaths occurred in June, one in July, and one in December. Of these, 3 showed evidence of hepatic disease, which represents a specific death rate of 1.03 per cent. after one exposure to *H. europaeum*. In the following summer of 1941-42, the plant was very sparse in the pasture although the rainfall appeared to be adequate. The death rate in 1942 rose to 5.2 per cent. Of the 15 sheep that died, 9 probably died from hepatic damage, giving a specific death rate of 3.1 per cent. This is a low figure compared with those for the second year in experiments B and C, but this second year period did not provide the same exposure to risk, since heliotrope was not available in any quantity to the sheep.

In 1943 the death rate rose to 50 per cent.; the great majority of the deaths were due to hepatic disease. In this year, as in experiments B and C, the sheep grazed the heliotrope mainly from January to April.

Because the plant growth is seasonal, we might expect a seasonal incidence in death rate. In 1941, after a first exposure to the plant, the specific deaths occurred in June, July, and December. In 1942, specific

deaths occurred, 1 in January, 1 in February, 5 in November, and 2 in December. In 1943, 81 of the 135 deaths occurred in May, June, and July; there was a secondary rise in November and December.

(ii) *Experiment B.*—There were 16 deaths, giving a total death rate of 11.6 per cent., during the first 12 months in the group which had access to heliotrope. The cause of death was not determined in all instances. Six of the animals died with well-advanced liver damage. In 4, the liver damage predisposed to the development of lesions of photosensitization which became infected and brought about death. Five of the carcasses were decomposed when recovered. In the control group, the death rate was 3.6 per cent. There is no reason to believe that in the heliotrope group the non-specific death rate would be less. It can be estimated on this basis that the specific death rate in the heliotrope group was approximately 7 per cent. during the 12 months after the first exposure to heliotrope. The incidence was highest in September-October, 4 months after the consumption of heliotrope ceased.

After the second exposure to heliotrope, there were 50 deaths during the period of 12 months, giving a total death rate of 41.0 per cent. It is estimated that the specific death rate was approximately 38 per cent. during this period of 12 months. The incidence was highest in May-June, about 2 months after the consumption of the plant had ceased.

(iii) *Experiment C.*—During the first 12 months there were 11 deaths in the heliotrope group, giving a total death rate of 7.6 per cent. It is estimated that the specific death rate was approximately 5 per cent. The incidence was highest in October-November, 4-5 months after the consumption of heliotrope had ceased.

After the second exposure to heliotrope there were 93 deaths during the period of 12 months, giving a total death rate of 70 per cent. All the animals examined showed advanced liver damage. Therefore the specific death rate is estimated to be approximately 70 per cent. Most of the deaths occurred from June to November, with the highest incidence in August-September.

It is clear from these results that a small proportion of the most highly susceptible sheep may die after the first year's exposure to the consumption of *H. europaeum*, but that two consecutive exposures are usually necessary to produce a lethal effect in sheep with a mean susceptibility. The results also show that the liver disease produced is progressive; that some degree of liver damage is carried over the period of 7 or 8 months from May to December which greatly increases susceptibility to a repeated exposure, and that both after a first and second exposure a period of 3-5 months elapses before the lethal effect becomes fully manifested.

These results are summarized in Table 3, in which the three separate experiments A to C are compared. In most of the mortality curves a peak

was reached between May and September, but a secondary rise occurred in November-December. In the table, the incidence of the primary peak only is indicated.

TABLE 3

COMPARISON OF SEASONAL INCIDENCE AND OF SPECIFIC DEATH RATE OF SHEEP WHICH CONSUMED *H. EUROPAEUM* IN THE THREE EXPERIMENTS

Experiment	After First Exposure		After Second Exposure	
	Specific Death Rate (approximate) (%)	Seasonal Mortality Peak	Specific Death Rate (approximate) (%)	Seasonal Mortality Peak
A	1	June-July	50*	May-July
B	7	Sept.-Oct.	38	May-June
C	5	Oct.-Nov.	70	July-Sept.

* Protracted exposure.

The results of experiment C confirm those of experiment B. The total death rate in the second year was higher than that in experiment B but the incidence of haemoglobinuria was much lower, and that of jaundice was appreciably lower in experiment C. The results are briefly summarized in Table 4. The data for the control group in experiment B are not given because the control of heliotrope consumption in this group was imperfect. The results obtained during experiment A are also disregarded.

TABLE 4

COMPARISON OF TOTAL DEATH RATE IN EXPERIMENTS B AND C; ALSO OF OCCURRENCE OF HAEMOGLOBINURIA AND OF HIGH LIVER-COPPER VALUES

Material with post-mortem decomposition excluded

Group	Experiment	First Year			Second Year		
		Total Death Rate (%)	Hb'uria (%)	Liver Cu 1000 p.p.m. or over (%)	Total Death Rate (%)	Hb'uria (%)	Liver Cu 1000 p.p.m. or over (%)
Heliotrope	B	11.6	18 (2/11)	45.4	41.0	31 (13/42)	83.3
	C	7.6	9 (1/11)	42.8	70.0	5 (3/60)	77.7
Control	C	3.5	Zero	Zero	4.8	Zero	Zero

III. PATHOLOGY

(a) Clinical Pathology and Biochemistry

The experimental studies showed that consumption of *H. europaeum* by sheep may kill a large proportion of the flock. However, the disease produced is somewhat protean in character although liver injury is responsible directly or indirectly for the diseased state.

To determine changes in blood and urine of affected sheep, some clinical and biochemical examinations were made. Despite obvious difficulties under field conditions, some information on the nature of the disturbances of metabolism was obtained.

At first, special attention was given to the study of changes in the level of blood copper which might be correlated with the occurrence of haemoglobinuria. Between June 17 and July 1, 1943, when the death rate in experiment A was at its height (*vide* Fig. 1), a daily sample of blood was collected from approximately 80 sheep remaining in the restricted-grazing group. During the period there were eight deaths but none of the animals developed clinical haemoglobinuria. These deaths were thus distinct from those occurring in outbreaks of chronic copper poisoning in sheep (*vide* Albiston *et al.* 1940; Bull 1951).

Examinations were also made of a small number of sheep from the flock, especially selected because they appeared to be ailing, during the period from May to July 1943, to be known as group A. Some attempt was made to correlate biochemical with clinical and histopathological findings.

A further group, group B, of 19 sheep was subjected to systematic examination between May 29 and July 29, 1944. The examinations were made to supplement those made on group A, although these sheep were the residue of the flock and were therefore the most resistant. The results of the examinations are briefly summarized as follows.

(i) *Blood Copper*.—Blood samples were collected daily from group A. Seven of the 24 sheep died on the day after selection, but from the others daily samples of blood were collected for periods ranging from 3 to 14 days. During the observations, 22 of the 24 died; 2 were discharged and died some time later. In all, 108 blood samples were examined from the 24 sheep. Most of the values fell between 0.8 and 1.2 $\mu\text{g/ml}$. In 2 sheep examined once only, the value was 2.3 $\mu\text{g/ml}$, but each died the following day. Subsequent histological examination of kidney sections showed the absence of haemoglobin casts and of the P.B. reaction in each case. Two other sheep had blood-copper values of 1.9 $\mu\text{g/ml}$.

One sheep showed normal values for 5 days. On the sixth day there was a slight rise to 1.6 $\mu\text{g/ml}$, and the values reached 3.7 $\mu\text{g/ml}$ at 7 and 8 days. The van den Bergh reaction became strong; the urine became dark brown and contained an appreciable amount of protein during these last 2 days of life. Histological examination of kidney sections showed some of the convoluted tubules to be dilated, and some to contain a slightly pigmented material resembling haemoglobin casts. Iron-containing granules were demonstrated in the epithelial cells of many of the convoluted tubules by the P.B. reaction. Blood haemoglobin fell from a normal level of 9.7 g/100 ml during the first 5 or 6 days to 7.9 g/100 ml on the eighth day. It seems probable that a small amount of haemoglobin was

passed through the glomerular tufts, but a frank haemoglobinuria did not develop.

Another sheep showed a blood-copper value of $3.2 \mu\text{g/ml}$ on the first day of examination. This fell to 2.7 the following day and reached 1.2 at 5 days when the animal died. The haemoglobin content fell from 9.8 to 7.4 g/100 ml over the same period. The van den Bergh reaction increased, and the blood urea rose to about threefold normal values. The urine changed little in colour from normal, but contained protein throughout. Histological examination of kidney sections showed absence of haemoglobin casts and of the P.B. reaction. Bile pigments were present on microscopic examination of liver sections.

These examinations thus showed that a slight degree of instability of the blood copper was present in some of the sheep, but a steep rise preceding the development of a frank haemoglobinuria was not demonstrated.

Between May 29 and July 29, 1944, when the death rate in the residue of the flock was falling, 19 sheep in group *B* were subjected to daily analysis of blood for copper. The period of observation ranged from 4 to 45 days, but the majority fell between 14 and 35 days. In all, 390 analyses were carried out. In 6 sheep the blood copper rose above normal to $2.0 \mu\text{g/ml}$ or over. In 3 of the sheep a value of between 2.0 and $2.2 \mu\text{g/ml}$ was obtained on a single occasion only. In one sheep the value reached was 3.0, but on the following 2 days it fell to 1.1 and on the third day it rose to $2.2 \mu\text{g/ml}$, after which it fell to normal again. Two sheep developed values of 3.7 and $3.8 \mu\text{g/ml}$ respectively, on single occasions only, but showed normal values immediately before and after the rise. Of the 19 sheep, 11 died and 8 were discharged at the end of the period. There was thus some slight degree of instability of the blood copper at this late stage in the history of the flock.

Subsequently, analyses were made of the blood from small groups of sheep grazing heliotrope, but no disturbance of the blood-copper levels was found in these.

(ii) *Blood Haemoglobin*.—Estimation of the haemoglobin content of the blood was carried out on 18 of the selected sheep in group *A*. Haemoglobin content was within normal range (8.5 to 11.0 g/100 ml) when first examined in 13 of these animals, but fell below normal in 4 within 3-5 days. It was below normal range in 5 when first examined, and remained low during a period of 6-10 days. One animal was distinctly anaemic when first seen; the blood contained 3.2 g/100 ml of haemoglobin. Over a period of 14 days the value rose to 5.5 g/100 ml . The sheep was then discharged but remained in poor condition; it died 9 months later.

In the remaining 4 of these 5 animals, the haemoglobin content of the blood at the first examination was 5.5 in one, 6.6 in two, and 7.6 g/100 ml in one. The value fell from 5.5 in the first of these to 3.2 in 4 days, rose to

6.3 on the 8th day but fell again to 4.4 g/100 ml on the 10th day, 24 hr before the animal died. This animal did not show haemoglobin casts in the kidney, but a strong positive P.B. reaction was present. It showed clinical jaundice but no bile pigment in liver sections.

(iii) *Haemoglobin in Plasma*.—Blood plasma was collected daily from the sheep in group B, and examined for free haemoglobin to detect any recognizable degree of *in vivo* haemolysis. Haemoglobin was observed in the plasma of 8 of the 19 sheep: on three occasions in 2, and on one occasion only in the other 6. In no instance was the haemoglobin present in large amount. The approximate range was from 0.25 to 0.5 g/100 ml but on one or two occasions it reached approximately 1 g/100 ml.

Of the two sheep in which free haemoglobin occurred on three occasions, one was under observation for 19 days, during which it was found on the 4th, 6th, and 12th days. The other was under observation for 37 days; haemoglobin was found in the plasma on the 22nd, 36th, and 37th days. Both sheep died and, although haemoglobin casts were not seen on microscopic examination of the kidney, a strong positive P.B. reaction was obtained in each. There were 3 other sheep which died soon after haemoglobin was observed in the plasma; a positive P.B. reaction was found in the kidney sections of each, along with a few haemoglobin casts in one.

(iv) *Van den Bergh Reaction*.—Sixty-four tests were carried out on the blood of 15 of the 24 animals in group A. An indirect reaction was obtained in 12 of these animals; 3 failed to develop a reaction. One of the 3 sheep which failed to develop a positive reaction died the following day, but another, which was the very anaemic sheep, remained clear of a reaction for 14 days after which it was discharged; a third remained clear up to the time of death on the 8th day.

Not all the animals giving a positive reaction showed clinical jaundice at the time. In 11 of the 24 animals under examination, clinical jaundice was present; in one it was very slight, and in 3 it developed towards the end of the animal's life. In one of these 3, the haemoglobin fell from 10.3 to 8.0 g/100 ml, a positive van den Bergh reaction developed, and the sheep died on the 12th day; much bile pigment was seen in microscopic sections of the liver, as well as a few haemoglobin casts, and a strong P.B. reaction, in the kidney. In most instances there was a correlation between the presence of clinical jaundice, of a positive van den Bergh reaction, and of bile pigment in sections of the liver. However, among the 11 sheep which showed clinical jaundice, 4 failed to show bile pigment in sections of the liver. Further, of the 22 sheep coming to autopsy during the period, 4 showed bile pigment in sections of the liver but no clinical jaundice.

In group B there were 19 sheep on which 382 van den Bergh tests were carried out during the period of 2 months, May to July 1944. Many

van den Bergh tests were carried out on other small groups. It was found generally that the sheep gave positive tests only during the last month or so of life, if at all. However, the liver could be extensively damaged, and lead to death, without the test becoming positive.

The evidence points to the jaundice being haematogenous in origin, that it is strongest when haemolysis has been severe enough to produce clinical haemoglobinuria, and weakest when evidence of passage of haemoglobin through glomerular tufts is absent. It was observed in these animals to wax and wane over short periods. The van den Bergh reaction has therefore proved a more reliable guide to the presence of the haematogenous jaundice than clinical observation.

TABLE 5
CORRELATION OF BLOOD-UREA VALUES FOR SHEEP OF GROUP A

Sheep	No. Days Observed up to Death	Blood Urea		Protein in Urine (day)	Blood Hb. Fall (g/100 ml)	Van den Bergh	Kidney Section	
		(day)	(mg/100 ml)				Hb. Casts	P.B. Reaction
1	5	4	170	— (1), — (2), ± (3), + (5)	..	..	—	+
2	8	1-6	Normal	++ (8)	9.7 to 7.9	+++	+	++
		7	64					
3*	5	8	129					
		2	90	++ (2)	9.8 to	+++	—	—
4	9	5	125	+ (5)	7.4	+++		
		1	59	± (6)	10.6 to		P.M. changes	
		7	86	+ (8)	6.8			
5	12			++ (9)				
		7	47	+	5.5 to 3.2	++	—	++
		8	63	—	to 6.3	—		
		9-12	50-60	++	to 4.4	+		
						++		

* This sheep had a blood-copper value of 3.2 $\mu\text{g/ml}$ on the first day; it fell gradually to 1.2 $\mu\text{g/ml}$ on the fifth day.

(v) *Blood Urea*.—The method used was that of Hench-Aldrich as modified by Fairley and Splatt (1926). The formula used for sheep was $40x - 50$, instead of $40x - 60$ as used for man. The normal range for sheep was 20 to 50 mg/100 ml.

Of the 24 sheep in group A, 9 were examined systematically 41 times. Higher than normal values were found in 5 animals only, all of which died. The results, correlated with other findings, are set out in Table 5.

The 4 remaining sheep had blood-urea values between 30 and 40 mg/100 ml. One of them had protein in the urine about the time of death on the 12th day; the blood urea was not determined then. Sections of the kidneys showed some haemoglobin casts and a positive P.B. reaction. The blood haemoglobin had fallen from 10.5 to 8.3 g/100 ml.

Blood-urea estimations were also carried out on 16 sheep in group *B* during a period of 45 days. Twelve of them had recently consumed heliotrope for the third annual period; 10 died and 2 were discharged. With the exception of the one discharged at 45 days, all showed some rise of blood urea above normal at some time during the period, usually a day or two before death, and all of these also showed a positive van den Bergh reaction. Four of them had not eaten heliotrope for about 14 months. Of these, 3 showed no rise in blood urea; the fourth showed a slight rise between the 10th and 16th day, but it remained normal until the sheep was discharged on the 32nd day. At the time of the rise in blood urea, a weak positive van den Bergh reaction developed and then disappeared.

(vi) *Urine Protein*.—Observations were made on the reaction and on the colour of the samples of urine in group *A*. The samples from 18 of the sheep were also tested for protein by the sulphosalicylic acid method qualitatively; the intensity of the reaction was some guide to relative values.

Usually a strong reaction was obtained a day or two before death. In the sheep which was very anaemic throughout the 14-day period but survived and was discharged, a trace only of protein was found on four occasions. Two other sheep showed traces only. One sheep under observation for 12 days before death, gave a strong positive reaction for the first 2 days, it decreased for the next 2 days, became completely negative for 2 days, then increased to a moderate reaction where it remained until the animal died on the 12th day. This improvement and relapse was correlated with the disappearance and recurrence of a positive van den Bergh reaction.

The correlation between urine protein and the passage of haemoglobin into the nephron is shown in Table 5.

(vii) *Pigments in Urine*.—The urine from a small number of sheep was examined for bile pigments. When a positive van den Bergh reaction was present in the blood, traces of bile pigments were usually present in the urine, but at no time did these become heavy in amount.

A special study of the pigments in the urine was not made. Spectrographic absorption curves constructed for urine samples from 6 of the sheep in group *B* yielded some evidence of the presence of porphyrins but the study was not taken further.

(viii) *Thymol Turbidity Test*.—The test was carried out on the sera of 19 ewes, part of the residue from the flock in experiment *A*. The method was that described by MacLagan (1944). In 9 normal control sheep, turbidity ranged from 0.6 to 1.6 units, with a mean of 1.0. In the 19 test ewes the range was from 0.8 to 3.8. Readings above the maximum of 1.6 for the controls were obtained in 8 of the 19 ewes.

(ix) *Plasma Phosphatase*.—The alkaline phosphatase activity in the plasma was estimated by the method of King and Armstrong (1934). The same two groups as those used for the previous test were examined. The highest figures were obtained with the control group. This result is not unexpected as the test is said by Higgins *et al.* (1944) to yield results above normal range mainly in obstructive jaundice.

(x) *Rate of Utilization of Propionate*.—A test was devised in which 200 mg of sodium propionate per kilogram of body weight was injected intravenously, and the rate of clearance of propionate from the blood, as

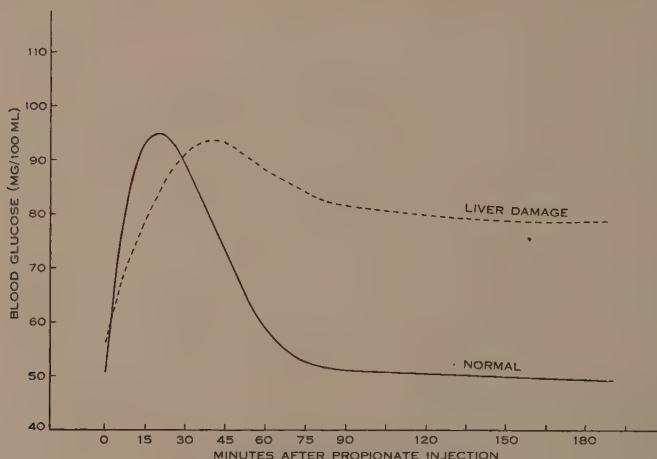


Fig. 2.—Rate of formation and clearance of glucose in the blood of sheep after intravenous injection of sodium propionate. Continuous line shows mean values for 16 normal sheep and broken line shows mean values for 8 sheep (group II) which had grazed *H. europaeum* for two consecutive seasons.

well as the rate of rise and clearance of blood glucose, were determined. The sheep were fasted for 24 hr prior to the injection. The sodium propionate, dissolved in 60 ml of water, was slowly injected into the jugular vein on one side of the neck over a period of 3 min, and blood samples for analysis were collected from the opposite jugular vein.

Total volatile fatty acids were determined by the method of McClendon (1944), and blood sugar by the method of Shaffer and Somogyi (1933).

In seven tests on 5 normal control sheep, the pre-injection values for volatile fatty acid were usually approximately 2.0 mg/100 ml, but covered a range of 0.7 to 4.0 mg/100 ml. Following the injection of 200 mg sodium propionate, all values returned to the pre-injection levels in 30-45 min. The same rate of clearance was found in 43 sheep which had grazed on heliotrope for one, two, or three successive seasons.

In the 5 normal control sheep, blood glucose rose to a maximum value of approximately twice the pre-injection level in 15-30 min and returned to that level, approximately, in 60-90 min. Approximately the same peak values were reached in sheep which had grazed heliotrope, but in 10 per cent. of them the rate was appreciably delayed, the peak being reached in 2 hr. In many of the others a delay of 15 min was recorded, the peak being reached in 30-45 min. The difference in response is illustrated in Figure 2.

In the study of the rate of glucose clearance in normal sheep, an approximation to the pre-injection levels was reached in 60-90 min. In sheep that had grazed heliotrope the rate of clearance was appreciably delayed (Fig. 2).

TABLE 6

THE BLOOD GLUCOSE VALUES AT 180 MIN AFTER INJECTION OF SODIUM PROPIONATE IN CONTROL SHEEP AND IN SHEEP THAT HAD GRAZED HELIOTROPE

Group	No. of Sheep	No. of Seasons on Heliotrope	Blood Glucose		Difference between Pre- and Post-Injection	
			Range (mg/100 ml)	Mean (mg/100 ml)	Range (mg/100 ml)	No. over 20 mg/100 ml
I	14	3	48.0-101.3	72.0	0-54	6/14 (43%)
II	8	2	65.1-101.4	82.8	7.3-46.9	5/8 (62%)
III	12	1	46.7-98.7	74.6	0-36.8	4/12 (33%)
IV	9	1	46.4-91.9	64.7	0-22.6	2/9 (22%)
Control	5	—	41.8-58.8	50.8	2.2-18.0	—

For comparative purposes, a time of 180 min after injection gave values which were useful. In the small group of normal sheep, the mean difference between the two levels was 10.6, and the maximum 18.0 mg/100 ml. If we regard the normal limit of the difference as 20, we find that a proportion of the sheep that had grazed heliotrope yielded results which exceeded this limit. The results are set out in Table 6.

The results do not show striking departures from normal. They probably indicate that there is no disturbance of metabolism of volatile fatty acid. Nevertheless, they seem to indicate some correlation between the delay in glucose clearance from the blood and the degree or stage of liver damage. It might be expected that the sheep in group I would show a wider departure from the normal range than those in group II. The reason for the difference being slightly smaller than that in group II may possibly be explained by the fact that group I consisted of a small residue of the most resistant sheep in the experimental flock used in experiment C. Further, they were grazing on the field station where heliotrope had not been abundant during their third exposure, whereas groups II and III had been grazing on a pure stand of the plant on an

irrigated block. There is a similar difference between the results in groups III and IV; group IV had been grazing on the natural pasture of the field station along with group I.

(xi) *Prothrombin in Plasma*.—In April 1947, Professor H. R. Carne carried out a prothrombin test on 50 control sheep and on 50 sheep that had had one exposure to heliotrope during experiment B. He found that 12 per cent. of the sheep in the heliotrope group had clotting times beyond normal limits.

We carried out some systematic tests on a group of 47 sheep which had their first access to heliotrope in February 1948. The method used was that of Quick as standardized by Aggeler *et al.* (1946). A pre-experimental control reading was obtained 10 days before they were given access to the plant. Two further tests were made, one in April and the other in May, during the period they were grazing on heliotrope. The results showed that the clotting time gradually became prolonged. At the end of the period of 3 months on the heliotrope, 8 per cent. of the group gave clotting times beyond the normal pre-experimental range. The proportion of the group falling into the several clotting-time periods at the three stages in the experiment is set out in Table 7.

TABLE 7
PERCENTAGE DISTRIBUTION OF CLOTTING TIMES IN A GROUP OF 47 SHEEP

Period on <i>H. europaeum</i>	Clotting Time (sec)					
	9.0 to 10.9	11.0 to 12.9	13.0 to 14.9	15.0 to 16.9	17.0 to 18.9	19.0 to 20.0
Pre-experimental	36	45	16	2	—	—
2 Months	—	37	54	9	—	—
3 Months	—	—	55	36	6	2

The results show a definite change in the level of prothrombin production although only a small proportion fall outside the normal pre-experimental range. This may be taken as an indication of a definite change in the efficiency of the metabolism of the liver cell, even at this early stage before the histopathological picture shows much more than a commencing megalocytosis.

During the same period there was no change in the fibrinogen content of the blood.

The test may be more valuable in the detection of a group change than in characterizing the more susceptible individuals. Other tests on sheep with more advanced liver damage from heliotrope failed to indicate that prothrombin production becomes seriously impaired, except perhaps as a terminal event.

(xii) *Serum Proteins*.—Determinations were made of the serum proteins of a small group of 20 of the more resistant sheep, which formed the residue from the heliotrope group in experiment C, when they had just started to graze heliotrope for the third time. The mean total protein was 5.9 g/100 ml, with a range of 4.4-8.0. The mean albumin was 2.5 g/100 ml, with a range of 1.2-3.5. The mean globulin was 3.3 g/100 ml, with a range of 2.0-6.8. The mean albumin:globulin (A/G) ratio was 0.73, with a range of 0.16-1.52.

Most of these sheep were in reasonably good health at the time, but one was in poor condition and died about 3 weeks later. This animal showed the lowest albumin of 1.2 g/100 ml, and the highest globulin of 6.8, giving an A/G ratio of 0.16, which was well below the mean of the group. The mean A/G ratio for a small group of normal sheep was found to be 1.4.

Further determinations were made on small groups consisting of between 5 and 20 sheep which had grazed heliotrope for a short period or had started on the second period of grazing. None of these sheep showed clinical evidence of disease at the time. The A/G ratio was somewhat lower than normal but not below 0.7 in any individual. The mean figures for the groups did not fall below 1.1.

The results indicate that, although the sheep that had grazed heliotrope twice or more possessed damaged livers, there was not a serious fall in serum albumin and in the A/G ratio until a fatal termination was approached.

(xiii) *Tests of Susceptibility to Chronic Copper Poisoning*.—One of the effects of the consumption of heliotrope by the sheep in the experimental flocks was an increase in copper concentration in the liver. Further, a small proportion of the sheep died following a haemolytic crisis similar to that of chronic copper poisoning in sheep. Field investigations of natural outbreaks of chronic copper poisoning in sheep in New South Wales showed that these outbreaks were commonly associated with liver damage resulting from prior consumption by the sheep of *H. europaeum*.

An experiment was carried out to determine if evidence could be obtained to show that consumption of heliotrope raised the susceptibility of sheep to chronic copper poisoning. Crossbred wethers were used. One group was allowed to graze natural pasture at the field station and to consume heliotrope grown with the aid of flood irrigation, as in experiment B. The other, which served as a control group, was grazed on improved pasture at a field station near Melbourne where heliotrope has never occurred. The copper concentration of the liver was determined on biopsy specimens collected by the method described by Dick (1944, 1952). The groups were chosen at random. By chance, 2 of the 16 sheep in the heliotrope group had liver-copper values between 900 and 1000 p.p.m. These were excluded from the main experiment. With this exclusion, the

group mean copper concentration of the liver was 356 p.p.m. The group mean for the 16 sheep in the control group was 262 p.p.m.

After 2 months' grazing on *H. europaeum*, the heliotrope group was brought to the laboratory where, along with the control group, the sheep were given by drench, over a period of 55 days, a dose of 10 mg copper on 36 occasions. The analysis of biopsy specimens taken within 9 days of the last dose of copper showed the mean copper concentration in the two groups to be 629 and 676 p.p.m. respectively. About 2 weeks later, 4 sheep in the heliotrope group and 6 sheep in the control group were killed for collection and examination of the livers; the mean copper concentration was approximately equal for the two groups. The body weight and liver weight were higher in the control group which had been grazing on improved pasture. Microscopic examination of liver sections showed slight but rather indefinite enlargement of the parenchyma cells in 2 of the 4 animals in the heliotrope group. It was obvious that the period of 2 months' consumption of heliotrope was rather short, and the degree of change in the liver cells less than that seen usually after a first exposure of 3 to 4 months.

The two sheep which were excluded from the main experiments, because of the initial high liver-copper concentration, were nevertheless passed through the experimental procedures with the others. They grazed the heliotrope, and were then subjected to the 36 doses of 10 mg copper. One died, after 30 doses of copper, from the haemolytic crisis of chronic copper poisoning. Microscopic examination showed well-advanced typical liver damage from the heliotrope in addition to central necrosis, and kidney damage from the haemoglobinuria. The other died 10 days after the completion of the administration of copper, but before the carcass was recovered post-mortem changes obscured the pathological picture. Nevertheless haemoglobinuria could definitely be excluded.

Seven months after grazing on heliotrope had ceased, the remaining 9 sheep in each group were subjected to further liver-biopsy examination. The sheep had remained at the laboratory, and were fed on a common diet of chaffed oats and lucerne hay. The examination showed a 50 per cent. fall in mean liver-copper concentration in the control group as against a 30 per cent. fall in the heliotrope group. The sheep of both groups were given another short course of copper by drench. Each received 30 daily doses of 10 mg copper.

The heliotrope group was returned to the field station, about the middle of January, where young heliotrope plants were present in the pasture. In February they were placed for the second time on an area of heliotrope which had been irrigated to ensure germination of seed. They remained there for a period of 2 months. After this they were returned to the laboratory, and subjected to the administration of 10 mg

copper per day for a period of 60 days. At the end of this period, examination of liver-biopsy samples showed the mean copper concentration of the liver in the heliotrope group to be 1077 p.p.m. whereas that in the control group was 702 p.p.m. One sheep in the heliotrope group died a few days before the administration of copper ceased. It died from advanced liver damage. Bile pigment was present in the liver, suggesting that some destruction of erythrocytes had occurred some time before, but a terminal haemoglobinuria did not develop.

Further biopsy samples of liver were taken 3 months later. The mean copper concentration in the heliotrope group had fallen approximately 15 per cent., that in the control group had fallen approximately 20 per cent. The sheep in both groups were then given a daily dose of 30 mg copper. After 67 days, one sheep in the heliotrope group died after developing the typical haemolytic crisis. Another in the same group died on the 102nd day, a third on the 106th day, and two more on the 112th day, all from the effects of the haemolytic crisis of chronic copper poisoning. The copper concentration in the liver of the 5 sheep was 2220, 3081, 2643, 1725, and 3390 p.p.m. respectively, expressed as copper to solids not fat. The total content of copper in the livers was 198, 260, 244, 224, and 250 mg respectively. The dosing procedure continued with the remaining sheep for a period of 138 days, but further deaths did not occur in either group.

The difference in the rate of storage of copper during the periods of copper dosage, the difference in the rate of copper loss from the liver during periods when no copper supplement was given, and the difference in the death rate, 63 per cent. in the heliotrope group as against zero in the control, support field evidence, and that obtained with the main experimental flocks, that consumption of *H. europaeum* increases the avidity of the liver cells for copper and increases the susceptibility of sheep to death from chronic copper poisoning.

(b) *Morbid Anatomy*

A study of the natural history of the disease in the three experimental flocks showed that a fatal termination might occur either from kidney damage or from liver damage, and that, for the most part, these deaths could be placed in one or other of three categories which are defined as follows: (i) In the first category are deaths from a severe haemoglobinaemia with consequent loss of kidney function. (ii) In the second category are the so-called sudden deaths that occurred in well-conditioned sheep usually about 2 or 3 months after the consumption of heliotrope had ceased in the second year of the experiments; these deaths went to form the peak in the mortality curves. (iii) In the third category are the deaths that occurred, frequently later, in sheep which usually had been losing weight and were obviously ailing.

The nature of the morbid changes in, and the naked-eye appearances of, the tissues varied broadly according to the occurrence of frank haemoglobinuria on the one hand, or to the rapidity of the disease process in the liver on the other hand. Nevertheless, liver damage was common to all.

(i) *External Examination of Carcass.*—Jaundice was present in a proportion of the animals. For example, of the 50 sheep dying in the second year of experiment B, 25 (50 per cent.) showed slight to severe jaundice clinically or at autopsy, or both; of the 93 sheep dying in the second year of experiment C, there were 83 which were examined adequately, and of these, 28 (33 per cent.) showed slight to severe jaundice. The most severe bile-pigment staining of the tissues was found in sheep with frank haemoglobinuria.

(ii) *Subcutaneous Tissues.*—These were slightly to intensely stained with bile pigments in 50 per cent. and 33 per cent. of the sheep dying in the second year of experiments B and C respectively. In well-conditioned animals there was a normal amount of fat present, but in poor animals it was sometimes almost completely absent. Oedema was not observed.

(iii) *Serous Cavities.*—Clear fluid was found frequently in the peritoneal and pleural cavities but infrequently in the pericardial cavity. Its colour often resembled that of serum, but sometimes it was much paler. If post-mortem changes were present, the fluid frequently became tinted with blood pigment. Fibrin was absent from the fluid except that in the pericardium which often contained a loose clot. In experiment B, during the second year and during the main rise in the mortality curve, 77 per cent. of the carcasses showed fluid in the peritoneal cavity, 50 per cent. in the pleural, and 10 per cent. in the pericardial cavity. During the decline of the mortality curve the figures were 57 per cent., 36 per cent., and zero respectively.

In experiment C, more attention was paid to the estimation of the quantity of fluid present. The amount in the peritoneal cavity varied from a very small increase up to 2.25 l. The amount in the pleural cavities was frequently approximately half that in the peritoneal cavity. The analysis of the occurrence of fluid in the serous cavities in this experimental flock is set out in Table 8.

(iv) *Liver.*—The sheep that consumed heliotrope showed some gross changes in the liver, the nature and degree of which depended largely on the passage of time and the amount of the plant consumed.

(1) *Weight.*—When the sheep had grazed heliotrope for a period of about 4 months, the liver was appreciably heavier than that of the controls.

The most reliable evidence of this came from the use of small pilot groups of sheep which were run during a 12-month period with each of the two main groups in experiment C. Groups were run for 4 months and then slaughtered, and replaced by new groups from the same reservoir flock. In addition to the 3 groups each with the

TABLE 8
THE OCCURRENCE AND AMOUNT OF FLUID IN THE SEROUS CAVITIES OF SHEEP DYING DURING SECOND YEAR: EXPERIMENT C
Percentage values in parenthesis and italics

Period of Mortality Curve	Number Examined	Fluid Present in Cavity					Fluid Absent All Cavities
		Peritoneal	Peritoneal Only	Peritoneal and Pleural	Pericardial	Amount Peritoneal > 500 ml < 500 ml	
Pre-maximal	10	5 (50)	0	5 (50)	1 (10)	2 (20) 3 (30)	5 (50)
Maximal	48	44 (91)	14 (30)	30 (62)	5 (10)	15 (31) 29 (60)	4 (8)
Post-maximal	23	21 (91)	5 (22)	15 (65)*	1 (4)	5 (22) 16 (70)	2 (9)

* One of the 23 animals had fluid in the pleural cavity only.

main heliotrope group and the control group respectively, a fourth group was run for the full 12-month period and then slaughtered. At slaughter, the livers of the sheep were collected for examination and chemical analysis. At the end of the first 4-month period, April to August, the mean liver weight of the 2 groups was 672 g with no significant difference between the groups. At the end of the second 4-month period, August to December, the mean weight of the liver was 642 g with no difference between the groups. At the end of the third 4-month period, December to April, the mean liver weight of the group with the control flock was 519 g, but that of the group with access to heliotrope was 927 g. The 2 groups which had run for the full 12-month period showed the same order of difference in liver weight, viz. 520 g as against 905 g.

The majority of the deaths were in sheep in the second year of the experiments. The weight of the liver varied appreciably in sheep dying during any one period of the mortality curve. One among several reasons for the variation in liver weight is the variation in the amount of fat present.

TABLE 9

WEIGHT OF LIVERS OF SHEEP DYING IN SECOND YEAR IN THE HELIOTROPE GROUPS OF EXPERIMENTS B AND C

Experiment	Period of Mortality Curve	Number of Livers	Weight Adjusted to 6% Fat		Unadjusted Weight		Fat Content Range (%)
			Range (g)	Mean (g)	Range (g)	Mean (g)	
B	Pre-maximal	2	360-393	376	360-410	385	5.2-10.2
	Maximal	26	230-632	416	230-890	484	1.5-42.5
	Post-maximal	9	150-420	285	150-420	285	2.7-6.5
		37					
C	Pre-maximal	7	135-230	185	135-230	185	0.8-12.0
	Maximal	26	211-564	385	215-620	407	3.5-20.3
	Post-maximal	19			115-590	263	2.5-9.6
		52	115-590	261			

Analyses for fat, as total ether-toluene extract, were made on a large proportion of the livers. The amount of fat in the liver of normal sheep usually ranges from 4 to 6 per cent. of the wet weight. The fat content of sheep dying in the heliotrope groups ranged up to 44.5 per cent. as the maximum. If liver weights, therefore, are to be compared with a view to obtaining an assessment of the hypertrophic or atrophic processes at work, it is necessary to correct the wet weight for fat content. For this purpose we have adjusted the weights of those above 6 per cent. fat to the 6 per cent. level. A small proportion fall below this level but their not being adjusted does not affect the comparison.

An analysis of the wet weight and fat content of livers of sheep dying during the second year in experiments B and C is given in Table 9.

The liver weight of the control sheep during the same period was between 500 and 600 g. The livers from sheep dying during the height of the mortality curve were heavier, and frequently more fatty, than those from sheep dying during the pre- and post-maximal periods. The correction of liver weights to a 6 per cent. fat level affects little the mean liver weight of the group, but in individual instances the weight of a fatty liver can be misleading. For example, the liver containing 42.5 per cent. of fat weighed 770 g, but adjusted, 499 g, i.e. slightly below control mean. The lowest weight recorded was 115 g, approximately one-fifth average normal weight for sheep of the same breed, type, and age.

(2) *Colour*.—Depending on the amount of fat present and the degree of bile-staining, the colour of the livers varied from approximately normal to bright golden yellow. In the atrophic livers of the more protracted cases, the colour was usually dull or pale, and greyish in hue.

(3) *General Appearance*.—Some livers appeared approximately normal although somewhat smaller than those from the controls. When there was much fat present, the liver appeared larger and the capsule more or less tense. In many instances the capsule was thickened, usually irregularly, but with no evidence of scarring. In the more protracted cases, the surface of the liver showed numerous scattered small lightly coloured miliary areas about 2-3 mm or less in diameter, usually very distinct because of their pale yellowish grey colour; they were distributed through the substance of the liver. Sometimes the surface of the liver was slightly raised over these areas. They were areas of regenerating liver tissue. In some sheep, killed a month or two after the completion of the 2-year experimental period, these areas of regeneration were larger and caused more irregularity in the surface of the organ. These changes in general appearance, when clearly evident, gave a striking picture, as illustrated in Plate 1, Figure 1.

(v) *Omentum*.—In the more emaciated animals there was usually a lack of omental fat, but no other abnormality was noted.

(vi) *Kidneys*.—A small proportion of the sheep died after developing a haemoglobinaemia. Often this was moderate to slight, but in a few instances it was severe, and then the kidneys were extremely dark in colour. *In situ*, the characteristic appearance was that of gun-metal. In less severe cases of haemoglobinuria the colour was not so deep. Especially in the instance of so-called sudden death, the kidneys were frequently affected with a severe vascular congestion. To differentiate the kidney with extreme vascular congestion from one containing free haemoglobin, a slice of kidney was placed in hypertonic saline solution; this would reveal the presence of free haemoglobin, if it were there.

(vii) *Urinary Bladder*.—The bladder was commonly empty from agonal contraction, but when haemoglobinaemia had occurred some red

to dark brown urine might be found. Otherwise there was no abnormality to be seen.

(viii) *Suprarenal Glands*.—These were free from abnormality.

(ix) *Spleen*.—Sometimes petechiae were to be seen on the surface.

(x) *Stomachs*.—The rumen, reticulum, omasum, and abomasum were usually free from abnormality.

(xi) *Small Intestine*.—Sometimes the mucous membrane was congested and there was some haemorrhagic extravasation in the lumen.

(xii) *Large Intestine*.—This was usually free from abnormality but the contents of the colon frequently were desiccated.

(xiii) *Heart*.—Petechial haemorrhages were frequently present in the subepicardial tissues, but usually did not occur elsewhere in the organ.

(xiv) *Lungs*.—The lungs were free from oedema and other abnormalities.

(c) *Histopathology*

Tissues were collected for microscopic examination from the liver and kidney of all carcasses in which post-mortem changes had not progressed too far. Whenever possible, animals were slaughtered when it was judged they were *in extremis*. For the most part, 10 per cent. formalin in buffered saline was the fixative used, but the formol-sublimate method was used in addition. Paraffin-embedded sections of liver were stained with haematoxylin followed by eosin, van Gieson, or Scharlach R. Weigert's method for fibrin, and silver methods for reticulin were also used. All of these except the silver methods were used as a routine. Other special methods which were used will be mentioned in appropriate places.

Liver

It was found that the general histopathological picture of changes in the liver following the consumption of *H. europaeum* by the sheep was very characteristic. The change which seemed to have the greatest pathognomonic significance was the enlargement of the parenchymal cell of the liver. Since characteristically all the liver cells were involved, the change was called a megalocytosis. Bull (1955) has shown that this megalocytosis of the parenchymal cells of the liver is characteristic of the pathogenic action of the heliotrope and other hepatotoxic alkaloids, such as those present in *Crotalaria retusa* and *Senecio cunninghamii*, and that it is probably pathognomonic for the liver disease produced by the toxic, or pathogenic, alkaloids of the pyrrolizidine group.

The natural development of the disease in the liver has been studied in the material available at various stages of the experiment.

The simplified histological picture of the liver of the sheep that died from the disease may be described as follows. Sections show the

parenchymal liver cells to be larger than normal with nuclei, also larger than normal, containing an open chromatin network. The cytoplasm takes acid stains well. In most instances there are obviously damaged liver cells present in the centrilobular position. The peripheral cells are better preserved. Usually there is no focal necrosis. The sinusoid spaces are not well in evidence, and there is usually little or no vascular congestion or haemorrhagic extravasation. The lobule is small but more or less regular; the columns of liver cells extending from the central vein to the periphery consist of relatively few, frequently as few as six, large cells. About the centre of the lobule are to be seen some large distended phagocytic cells containing a ceroid material. A few of these cells may be seen in other parts of the lobule and also in the portal tracts. Globules, usually staining strongly with eosin, are to be seen in some of the liver cells, usually more to the periphery of the lobule. These vary considerably in size but usually from about 2 to 30 μ or more, and frequently occur in clusters. They stain intensely with Weigert's fibrin stain, and are thus more easily seen and differentiated by this method. There is usually much new bile duct formation in the periphery of the lobule, but there is slight, if any, increase in collagen formation associated with this hyperplastic change. The main increase in supporting tissue consists of reticulin. The capsule of the organ is usually thickened with new collagen formation.

It may be appropriate to recall that we are dealing with pathological changes which have occurred under particular circumstances within the limited time of 2 years in the main experiments. Shorter repeated exposures to the plant over longer periods of time, similar to those which may occur under field conditions, would produce a picture differing in some respects from that described. A more complete study helped to reveal the significance of and variation in some of the major histopathological changes. These are outlined as follows.

(i) *Megalocytosis*.—The nature of this change has been described by Bull (1955). It was found to be the primary and most pathognomonic change. It was observed to occur in the liver of sheep which had consumed the plant for a relatively short time (Plate 1, Fig. 2), e.g. 4 months (*vide* details presented on liver weight); it was not associated with appreciable change in the normal function. At a later stage, in sheep that died from the disease, the cells were larger still with much larger nuclei, some of them being gigantic. Megalocytosis was usually obvious, but in some individuals the cells were larger than in others. It was not so obvious or easy to recognize in livers which contained a large amount of fat, or in the livers with appreciable diffuse regeneration of cells.

The increase in the size of the cells has been determined by the method of Chalkley (1943). The mean cell diameter of normal liver cells of sheep has a range of 18.2 to 23.3 μ , according to the nutritional state of the animal. The megalocytic change is represented by an increase in cell

diameter beyond $23.3\ \mu$. This has been found to lie between $18.2\ \mu$ multiplied by from 1.4 to 3.0, giving a corresponding increase of cell volume.

(ii) *Fatty Metamorphosis*.—Microscopic examination of frozen sections of the liver of sheep that had died as a result of liver damage usually showed some fatty changes. There was sometimes evidence of some terminal damage to cells, particularly in the centrilobular position. In some instances, only a few individual cells showed accumulation of fat in the cytoplasm. In other instances the majority of cells in the lobule showed much accumulation of fat.

A random sample of 29 livers from sheep dying from the disease in experiment B were subjected to chemical analysis for fat. The fat content ranged from about 2 to 42.5 per cent. There was a positive correlation between the amount of fat in the liver and the body weight of the animal; the liver cells were unable to metabolize lipid brought to them mainly from the fat depots. The cells containing the fat were large and foamy in appearance in paraffin sections although sometimes large globules of fat were also present. Occasionally a vesiculated cell would be found to be due to an hydropic change.

In the chemical analysis of the 29 livers, 27 per cent. contained less than 6.0 per cent. of fat, 45 per cent. between 6.1 and 14.0 per cent., 10 per cent. between 14.1 and 30.0 per cent., and 17 per cent. between 30.1 and 42.5 per cent. Chemical analysis of 48 livers in experiment C showed that 43.7 per cent. contained less than 6.0 per cent. of fat, 43.7 per cent. between 6.1 and 14.0 per cent., and 12.5 per cent. between 14.1 and 20.3 per cent. There was usually a fairly close correlation between the microscopic and the chemical findings, although in the livers that gave low values for fat content, some of the cells would show some fatty metamorphosis on microscopic examination.

Livers collected from sheep slaughtered during the progress of the experiment showed no fatty metamorphosis. Therefore the process is regarded as terminal in character.

(iii) *New Bileduct Formation*.—This was a constant occurrence. It was present early in the disease; it was found in animals that were killed before any clinical abnormalities were detected. As liver-cell destruction continued, with the reduction in size of the lobule, new bileduct formation progressed. It was sometimes accompanied by round-cell accumulation in the portal tracts. It was associated with an increase of supporting tissue consisting of reticulin, but there was usually little increase in collagen. In a few instances the bileduct hyperplasia led to an adenomatous formation, but this was rare.

(iv) *Ceroid*.—A lipid material, which is microscopically identical with ceroid as first described by Lillie, Daft, and Sebrell (1941), was seen most clearly in large phagocytes, in coarsely globular form. Finer granules of material giving the same staining reactions were to be seen in some of the liver cells, when its presence elsewhere was more or less abundant. It was found in all the livers of the sheep that died of the disease, with the exception of a few that died early in the experiments. It was more abundant in the more chronic cases. The material has varied considerably

in abundance, but its presence is strongly indicative of heliotrope hepatitis in sheep, according to experience up to the present time.

The large mononuclear phagocytes which contain the material were usually distended to capacity, and appeared to be immobilized. They probably originated from Kupffer cells. The material was apparently liberated from dead liver cells, was not readily dispersed, and was taken up by the phagocytes which in turn failed to digest it. These ceroid-containing phagocytes were situated mainly about the centre of the lobule (see Plate 2, Fig. 1) but sometimes were found in the portal tracts.

The ceroid gives the main staining reactions and microscopic characteristics of the ceroid in the livers of choline-deficient rats, as described by Endicott and Lillie (1944). It has a pale yellow colour, stains with many basic dyes, with sudan dyes, and with Nile blue, is acid-fast by the carbol-fuchsin method (see Plate 2, Fig. 2), does not retain the stain in Weigert's fibrin method, but reduces silver with Laidlaw's method, does not react for iron in the Prussian blue or in the Turnbull blue methods, and it is autofluorescent both in paraffin and in frozen sections with ultraviolet and with blue light. The fluorescence is not quite so strong in our preparations as that of the ceroid in the rat liver kindly supplied to us by Dr. R. D. Lillie. The significance of this difference has not been investigated, but it is of no practical importance in determining the microscopic characteristics of the material.

(v) *Inclusion Globules*.—These globules, which have been described by Bull (1955), were present in the majority of the livers from sheep that died of the disease. They were difficult or impossible to detect in material that had undergone post-mortem decomposition. They were not found in the livers of sheep killed during the progress of the experiments. The evidence indicates that their occurrence is associated with the terminal stages of the disease. They sometimes occurred in large numbers and in large clusters (see Plate 3, Fig. 1), but at other times there were very few to be seen in a section. They were found most frequently towards the periphery of the lobule, and were rarely present in the more damaged cells around the centre. Sometimes they appeared to be extracellular, suggesting that one or more cells containing them had died, but the globules had failed to disperse. In tissue collected immediately after death they were seen to stain intensely with eosin. With Weigert's method for fibrin they retained the dye intensely, as they did also with Mallory's phosphotungstic haematoxylin method for mitochondria. There was some variation in the intensity with which some of the globules, particularly the larger ones, retained the dye: some of the larger globules appeared to have lost substance. With Mallory's method for mitochondria, a refractile unstained margin was detected in some of the globules. They agree in their staining reactions with inclusion globules described by Doniach and Weinbren (1952) in the cells of the rat's liver after hepatectomy, but their presence

in large clusters and their less ephemeral character present some points of difference. They are probably not entirely homogeneous, but may consist mainly of plasma albumen, as Doniach and Weinbren suggested for their globules, with some lipid constituent. They also seem to be identical with the cytoplasmic cell inclusions described by Fisher and Fisher (1954).

(vi) *Fibrosis*.—Fibrosis was not found to be a distinctive feature of the pathological changes. There was frequently a thickening of the capsule of the organ with well-developed collagen. In the portal tracts, associated with the new bileduct formation, there was frequently some formation of fine, somewhat immature, collagen along with a pronounced increase in reticulin fibrils. Single liver cells, or small groups of two or three, were sometimes seen isolated by new connective tissue fibrils at the periphery of the lobule. The normal argyrophil network of reticulin fibrils supporting regular walls or columns of liver cells was usually completely destroyed or disrupted. It was represented by irregular broken fibrils, sometimes of several thicknesses, usually short, which separated groups of cells. There was slight increase of reticulin fibrils around the central vein with sometimes short strands extending into the atrophic lobule.

(vii) *Haemorrhagic Extravasation*.—For the most part, the pathological changes were characterized by the absence of haemorrhagic extravasation. In a very small number of instances there were small, microscopic extravasations, usually centrilobular in distribution. These appeared to be terminal, and to have resulted from an overflow of blood into small spaces caused by the death of liver cells. Old blood pigment, which might have indicated earlier haemorrhages in the tissue, was consistently absent.

(viii) *Regeneration of Liver Tissue*.—Under the conditions of the experiments, the animals that died during the second year usually died from a fulminating liver damage characterized by the absence of regeneration of liver cells. Nevertheless, in some instances there were small miliary areas of recent regeneration. In other instances, microscopic examination revealed some diffuse spread of regenerating cells. These cells were always of small normal size, and frequently contained fat globules, even when the adjacent megalocytic cells contained none.

(ix) *Bile Pigment*.—Bile pigment was frequently to be found on microscopic examination. It varied from small amounts, which had to be searched for carefully, to large amounts which were present in all fields of the microscope. It was seen mainly in the canaliculi, but when present in large amounts occurred in many irregular masses. On rare occasions, bile pigment was observed in some of the bile capillaries in the portal tracts. Its detection was not appreciably affected by post-mortem decomposition. Special staining methods were not used; reliance was placed on the examination of unstained sections although it was found to be more readily seen in sections stained with malachite green. It was

found in some animals that showed no clinical signs of jaundice at the time of death. For example, it was found in 24 of 61 sheep (39 per cent.) that died in the second year of experiment B, but only 12 of the 24 sheep showed clinical signs of jaundice at the time of death. In no instance did the microscopic picture suggest the presence of an obstructive jaundice.

(x) *Microscopic Appearances in Resistant Recovered Sheep.*—The livers of sheep remaining from the control and heliotrope groups of experiment B were collected at slaughter 8 months after the conclusion of the experiment. The sheep remaining alive from the heliotrope group represented the more resistant animals, but the liver damage in some of them would probably have approached a lethal character at some time during the preceding 12 months. Heliotrope had not been consumed by the sheep for 16 months except for a very small and insignificant picking in December 1945, 9 months before slaughter. There were 64 sheep residue of the heliotrope group, and 117 from the control group, sent to slaughter. The livers were collected and weighed, and tissue was preserved for microscopic examination.

The outstanding findings were the continued existence of abnormally large liver cells and the presence of much regeneration. Phagocytic cells containing ceroid were not found in any of the sections. Appreciable fibrosis was present in 12 of the 64 livers (19 per cent.) from the heliotrope group. The fibrosis was interlobular in distribution (see Plate 3, Fig. 2). It consisted of collagen with some reticulin fibrils intermixed. In 21 (33 per cent.) there was no degree of fibrosis at all. In the remaining 31 (48 per cent.) there was some slight increase of collagen in the portal tracts, but insufficient to constitute a true fibrosis of the organ. In some instances there was some slight residue of adventitious bile duct formation.

In two or three of the livers there was little or no evidence of active regeneration of liver cells, but in most of them regeneration was very active and widespread. In some instances the regeneration appeared diffuse, but usually it was most apparent in the centre of the lobule, giving the larger megalocytic cells a peripheral distribution. In other instances it was scattered, focal, and distinctly miliary in character. When regeneration was well advanced, the reticulin network in the lobule was normal in appearance, but in the earlier stages it was imperfect. The lobules in most of the livers approached normal size. That regeneration of liver tissue was not complete was supported by the somewhat lower mean weight of the livers from the heliotrope group, which was 597 g as against a mean liver weight of 728 g from the control group.

Kidney

There were no microscopic changes found in the kidney in any way comparable with those found in the liver. The changes found were secondary in nature. The most outstanding in severity were those

secondary to haemoglobinaemia which, however, did not usually reach the severity commonly seen in the haemolytic crisis of chronic copper poisoning. In a few instances, in experiments A and B, the haemoglobinuria was severe. In these, most of the nephrons of the kidney were filled with haemoglobin and its degradation products. The proximal convoluted tubules were filled, and frequently widely distended, with haemoglobin casts in varying stages of disintegration. The epithelial cells of these tubules contained small globules of haemoglobin which was soon broken down to an iron-containing substance with the characteristics of haemosiderin, giving a positive P.B. reaction. The casts in the tubules appeared to lose their haemoglobin quickly, and remained as more or less pale-staining hyaline casts. At no time was there any production within the lumen of the tubules of haemosiderin or other product giving a positive P.B. reaction. In a few instances in which the amount of haemoglobin passed through the glomerulus was not gross, the contents of the tubules were granular and retained more of the blood pigment. The evidence suggested that, in these instances, death of the animal was not so rapid, and the reaction of the urine had changed from alkaline to acid.

For the most part, however, when haemoglobin casts were present their number was small, sometimes very small. In some instances a few were found in the collecting tubules only. Post-mortem changes in the kidney rendered microscopic examination of sections unsatisfactory. There were 55 satisfactory specimens in experiment A from the 135 animals that died; 11 (20 per cent.) showed the presence of haemoglobin casts in larger or smaller numbers. In experiment B there were 30 satisfactory specimens from 50 animals; haemoglobin casts occurred in 12 (40 per cent.). In experiment C there were 52 satisfactory specimens from 93 animals; haemoglobin casts were found in 4 (7.6 per cent.).

In all the sections showing larger or smaller numbers of haemoglobin casts, a strong positive P.B. reaction was obtained in the epithelial cells of the convoluted tubules. The reaction was strongest when the amount of haemoglobin passed through the glomerulus was largest. In many additional kidney sections, this P.B. reaction was obtained in the absence of haemoglobin casts. The intensity of the reaction graded down to zero. On all the evidence, including the absence of the reaction in other tissues, except the spleen, its presence in these kidneys must be taken as an indication of the passage of haemoglobin, even in small amounts, through the glomerulus. If the occurrence of haemoglobin casts, with the associated P.B. reaction, and that of the P.B. reaction in the absence of the casts are taken together, as indicating destruction of red blood cells and the passage of haemoglobin through the glomerulus, it is found that in experiment B 24 (80 per cent.) of the sections more or less free from post-mortem decomposition gave a P.B. reaction, and in experiment C there were 30 (57.7 per cent.).

The histopathological examinations also revealed other changes of less significance. The most interesting of these was the occurrence of dilatation of portions of the proximal convoluted tubules which appeared to have resulted from the previous occurrence of haemoglobin casts which had disappeared. In some of the kidneys there was a well-developed vascular congestion without haemorrhagic extravasation, but it was not frequent enough to be characteristic. In many of the sections there were cast cells in the subcapsular space of the renal corpuscles. These were cast from the epithelium of Bowman's capsule. This appeared to be largely, if not entirely, a terminal occurrence. Sometimes an occasional hyaline cast was found in some portion of the nephron.

IV. DISCUSSION

H. europaeum is a summer-growing herbaceous plant (see Plate 4). It has several common names, including heliotrope, caterpillar weed, and potato weed. It was probably introduced into Australia in the early days of settlement, but has spread widely in south-eastern Australia during the last 30 years. This has been favoured by the extension of the arable areas. It has not been found in the subtropical and tropical areas of Australia.*

When these experiments were started there was no evidence in the literature that *H. europaeum* was harmful to sheep. Pammel (1911) records that it contains a toxic alkaloid, heliotropine, and that it is regarded as a poisonous plant. In Australia it was suspected by some observers of having some harmful properties; that it was associated with the occurrence of "yellows" or toxæmic jaundice in sheep. Extensive tests, at the Veterinary Research Station of the Department of Agriculture of New South Wales, failed to reveal any toxicity of the plant for sheep (Hurst 1942). In these tests, sheep were fed on the plant for a period of 3 months. There was no suspicion that the plant contained hepatotoxic alkaloids. Therefore the feeding trials were carried out over a period of time inadequate to produce chronic effects.

Menshikov (1932) isolated and identified two alkaloids, lasiocarpine and heliotrine, from *Heliotropium lasiocarpum*. This work was unknown to us when our experiments were started, but during their course we obtained plant material of *H. europaeum* for chemical analysis. Trautner and Neufeld (1949) examined this, and recorded the isolation and identification of lasiocarpine and heliotrine. Later, Culvenor, Drummond, and Price (1954) and Culvenor (1954) reported the results of a more complete study of the alkaloid content of the plant. They found the two major alkaloids and their N-oxides, as well as three others and their N-oxides.

* Since this paper went to press C. W. E. Moore has published "Observations on the Autecology of *Heliotropium europaeum* L. in New South Wales and Victoria" (Tech. Pap. C.S.I.R.O. Aust. Div. Plant Ind. No. 7).

The present experiments prove beyond any doubt that consumption of the plant by sheep will produce a fatal liver damage provided a sufficient quantity is consumed over a long enough period of time. The toxicity of the alkaloids for rats has been studied; the results will be published elsewhere. In view of the moderately high toxicity revealed, it is remarkable that most sheep can, as a first experience, graze a pure stand of the plant for a period of about 4 months without obvious injury. This has been found in field observations, and is amply confirmed in the experiments. A change in the character of the liver cells occurs, and a small proportion, about 1 per cent., of the animals may die from liver damage 2 or 3 months after the consumption of the plant has ceased. Even when the grazing experience is repeated with the same sheep in the following summer, lethal effects do not become manifest until well after the consumption of the plant has ceased. The modification of the liver cells as a result of the first consumption persists for some time, and is a very striking event, particularly in view of the lapse of time, about 9 months, between the first and second exposures to the plant. It is believed that the relatively low toxicity of the plant, which can be eaten by the sheep in large quantities, is in large part due to the appreciable destruction of the alkaloids by the plant and bacterial enzymes in the rumen. If this suggestion is sound, only a small proportion of the alkaloid consumed may reach the abomasum unchanged, and be subjected to extraction and solution by the hydrochloric acid secreted there. During this period of retention of the plant material in the rumen, the N-oxides of the alkaloids, which are more readily soluble in water, may pass from the rumen more rapidly, and thus escape more or less successfully the destructive action of the bacterial and plant enzymes. The N-oxides are capable of being absorbed and of being reduced to the more toxic form in the tissues of the animal (unpublished results). It is possible, therefore, that much of the toxicity of the plant to the grazing ruminant is referable to its content of N-oxides of the alkaloids. It is pertinent to speculate on the possible reason for the great resistance of sheep to the consumption over several years of *Senecio jacobaea* L., which contains potent hepatotoxic alkaloids. Bradbury and Culvenor (1954) have found that it has little or no N-oxide content. Possibly the alkaloids are almost completely destroyed in the rumen and, in the absence of N-oxides, the amount of hepatotoxic substance absorbed is inadequate to produce pathogenic effects. A small proportion of sheep are susceptible, but in the majority, the small changes in the liver cells do not lead to a lethal effect.

Probably the first outbreak of heliotrope poisoning of sheep investigated in Australia occurred in a semi-arid area of South Australia in 1925 in Merino sheep. Bull (1928) reported this as an atrophic hepatitis possibly due to chronic phosphorus poisoning. A hepatotoxic poison plant was suspected. "Potato weed" (*H. europaeum*), unknown then as a poison

plant, was present during the previous two summer periods. Arrangements to collect the plant for feeding experiments failed. A re-examination of the history of the outbreak, and of the histological preparations, leaves no doubt that consumption of *H. europaeum* caused the disease.

During the course of the experimental investigation recorded here, investigation of outbreaks of so-called toxaemic jaundice and of chronic copper poisoning in the field have continued concurrently. Histopathological examination of liver and of kidney has enabled a correct diagnosis to be made in all instances. The experimental results have thus been confirmed. Nevertheless the final incrimination of *H. europaeum* as the cause of the liver damage in natural outbreaks depends on the confirmation of its presence in the environment. Other plants containing pyrrolizidine alkaloids may produce, in sheep, liver damage indistinguishable from that produced by heliotrope. For example, in at least one outbreak *Echium plantagineum* L. was incriminated; Crowley *et al.* (unpublished data) found pyrrolizidine alkaloids present in the plant.

Many outbreaks, and many deaths in sheep, have been due to the haemolytic crisis of chronic copper poisoning. The consumption of *H. europaeum* has predisposed sheep to this disease; death has not been due to advanced liver damage, but some damage with megalocytosis of liver cells has been characteristic of these particular outbreaks. This complex of heliotrope-chronic-copper poisoning has produced the majority of the outbreaks in New South Wales characterized by the sudden development of jaundice and death. Outbreaks of heliotrope poisoning have probably become less common in recent years as knowledge of the harmful nature of the plant has extended. The disease, in either of its two main manifestations, is very rare or almost completely absent in Merino sheep in New South Wales.

In South Australia since 1925, there has been one serious outbreak of heliotrope poisoning in Merino sheep on a property in the Flinders Range, a little further north from the area of the first outbreak investigated in that State. In neither instance was jaundice observed as a clinical sign of the disease, which was characterized by increasing loss of weight and cachexia. More recently, *H. europaeum* has spread widely in the agricultural areas just north of Adelaide. Its consumption by Merino and other breeds of sheep has produced liver damage and a photosensitization. Death in an appreciable proportion of the animals has resulted from lesions about the lips and face (Keogh 1955).

In the experiments under controlled conditions, the several disease manifestations seen in field outbreaks were expressed in larger or smaller degree. For example, some photosensitization occurred in the sheep under experiment. The degree of haematogenous jaundice was less in the experimental flocks than in many outbreaks in New South Wales, but higher than in most outbreaks in South Australia.

A high copper concentration in the livers of sheep that have consumed *H. europaeum* was as clearly manifest in the experimental flocks as under natural conditions in the field. Although the concentration of copper within the individual liver cells may reach dangerous levels, chemical analysis of a dried specimen of liver fails to give critical information on the amount of copper in available form per cell. Rubeanic acid treatment of microscopic sections has failed to show a correlation between the amount of copper in the cell and the total copper concentration determined by chemical analysis. This is probably determined by the manner in which the copper is bound. The more firmly it is bound the more difficult it becomes to demonstrate it with rubeanic acid in sections, and the less readily it is available for physiological needs. Experiments with rats, repeatedly injected intraperitoneally over a period of 3 months with small sublethal doses of the pure alkaloids, have shown (unpublished data) that the concentration of copper in the liver may be increased very considerably, up to one hundredfold. There is little doubt therefore of this particular effect of the action of the alkaloids on the liver cell. The haemolytic crisis of so-called chronic copper poisoning of sheep and its association with high concentration of copper in the liver are now well known.

The pyrrolizidine alkaloids of other plants have been found to have a similar action. The description of seneciosis in sheep in New Zealand by Gilruth (1905) gives a clear picture of well-conditioned sheep dying as a result of haemoglobinaemia with clinical signs of jaundice and haemoglobinuria, although the true character and significance of the terminal crisis appear to have been missed. We have also seen liver damage with a terminal haemolytic crisis in sheep that have consumed, over two or more seasons, *Echium plantagineum*, a member of the family Boraginaceae.

The exact part played by copper in the haemolytic process is under investigation, but has not yet been elucidated. The red blood cells are not permeable to cupric ions (Keilin and Hartree 1946). With *in vitro* tests copper salts cause the formation of methaemoglobin and agglutination of the cells, if low concentrations are used in a well-buffered system, but lysis of the cells does not occur. Nevertheless, Nossal (1949) has recorded the occurrence of lysis of rabbit erythrocytes in his study of the inhibition of cell respiration catalyzed by methylene blue. It seems that copper may have a direct effect in the production of lysis of mammalian erythrocytes if it is present in some combination which can penetrate the cell.

It is clear that copper plays either a direct or an indirect part in the lysis of the erythrocytes of the sheep, and that the haematogenous jaundice in sheep affected with the heliotrope hepatitis is due to altered copper metabolism and the high concentration of copper in the liver cells. The evidence from the experimental studies shows that haemolysis may vary from a slight recurrent and often terminal manifestation, to a severe

and lethal crisis. When jaundice occurs it appears to be invariably haematogenous in origin.

The pathological studies have shown that the disease produced by the consumption of *H. europaeum* is essentially one due to the altered metabolism of the liver cell. There is evidence, both morphological and biochemical, of this change in the liver cell even when the animal has consumed the plant over one short season, and has remained in good health with increasing body weight. At this stage there is usually no fall in blood haemoglobin and no occurrence of bile pigments to give a positive van den Bergh reaction, but there may be a slight change in prothrombin values and in glucose utilization from propionate. Nevertheless the animal may be predisposed to the development of the haemolytic crisis, particularly if it is placed to graze on a pasture, such as subterranean clover, favouring a high normal intake of copper. Thus the consumption of heliotrope may lead, and does so frequently under field conditions, to a failure of kidney function in which the cause of death is quite distinct from that in cases with advanced liver damage.

With continued or repeated consumption of the plant, the changes in the metabolism of the liver cell become greater. These lead to a higher death rate of the liver cells, and the organ becomes small or atrophic. The animal loses weight. There may be destruction of erythrocytes leading to a fall in blood haemoglobin values, the occurrence of bile pigments in the blood, a fall in prothrombin values, and a fall in plasma albumen, as well as some change in carbohydrate metabolism. The changed or damaged liver cells also lose some ability to deal with lipids with the result that ceroid is formed and accumulates in the liver tissues. The presence of ceroid is probably associated with a high consumption of the seeds, which contain much fat with a high content of unsaturated fatty acids. The less obviously damaged perilobular liver cells show the presence of protein inclusion globules. The presence of these globules suggests that although the cell is abnormal it retains the capacity to perform many, if not all, of its normal functions. For example, it may produce prothrombin and serum albumen, but the mechanism by which these are removed and go to maintain normal levels in the circulating blood breaks down. In the end, the animal may die because the vital function of the liver fails. Under these conditions the animal becomes weak and cachectic before death.

On the other hand, the animal may die more or less suddenly while still in good bodily condition. Deaths of this type occurred in the experiments when the pasture plants were young and vigorous, in the early spring, a few months after consumption of heliotrope had ceased. This type of death was distinct from that seen in animals with progressive cachexia, and from that seen in animals with haemoglobinaemia. On clinical and pathological evidence it is regarded as an acute toxic death. It is suggested that the progressive liver damage enabled the organ to deal with only a

limited nitrogen intake and turnover. With the increased nitrogen intake from the lush pastures there was probably an inability of the liver to convert ammonia into urea, and the animal died from acute ammonia intoxication. Unfortunately this suggestion is unsupported by determinations of blood-ammonium levels, but ammonia intoxication has been found under similar circumstances which will be reported elsewhere.

The experiments gave no evidence of nutritional factors playing any aetiological role in the progressive liver damage. However, there is some experimental and much field evidence to show that a falling plane of nutrition, particularly a fall from a high plane rather than a fall from a low to a lower plane, is liable to precipitate a haemolytic crisis especially in the earlier stages of the disease.

The results of the experimental investigations are presented in the belief that not only do they establish the pathogenicity of *H. europaeum* for sheep and present a picture of the essential pathology of the disease produced, but in addition they add to knowledge of the pathogenic action of the pyrrolizidine alkaloids in general.

V. ACKNOWLEDGMENTS

The results recorded in this paper were obtained from part of the work carried out during the course of the cooperative investigation of the disease of sheep known as toxaemic or enzootic jaundice. To provide the necessary cooperation and coordination of effort, the "Toxaemic Jaundice Investigation Committee" was formed in 1938. The constitution and membership of this Committee are given in the first published report on the investigation (Albiston *et al.* 1940).

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EXPLANATION OF PLATES 1-4

PLATE 1

Fig. 1.—Photograph of liver of crossbred wether which was affected with heliotrope hepatosis and died. Weight 480 g. Numerous circumscribed pale areas of regenerating liver tissue. The regeneration is more advanced, and the miliary areas are larger, than was usually found in the experimental sheep that died from the disease. $\times \frac{1}{2}$ approx.

Fig. 2.—Photomicrograph of section of liver of sheep slaughtered after it had grazed heliotrope for a period of about 3 months. The liver was heavier than those of the control group. The picture is that of early generalized megalocytosis of the liver cells. Inset, area of normal cells from control. $\times 120$.

PLATE 2

- Fig. 1.—Photomicrograph of section of liver showing ceroid-containing phagocytes about the central vein. Stain, Nile blue. $\times 450$.
- Fig. 2.—Photomicrograph of section of liver showing ceroid-containing phagocytes about the centre of the lobule which is very atrophic. Stain, Ziehl-Neelsen acid-fast method. $\times 120$.

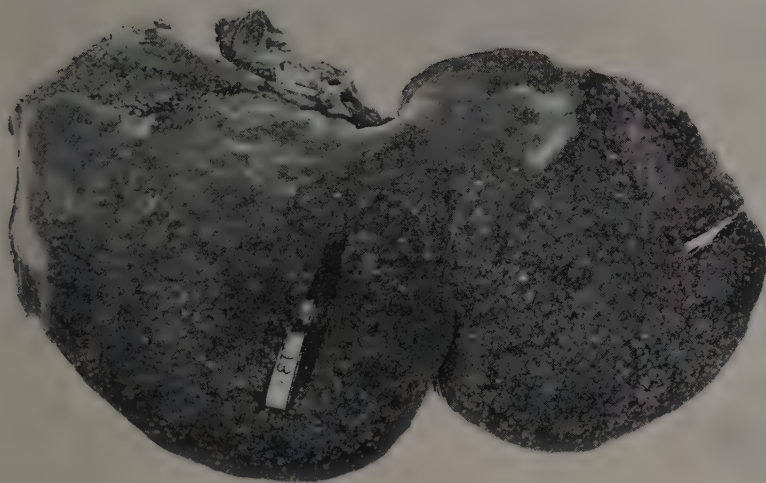
PLATE 3

- Fig. 1.—Photomicrograph of section of liver showing inclusion globules. Stain, Mann's. $\times 400$.
- Fig. 2.—Photomicrograph of section of liver of sheep that had recovered from active liver damage in experiment B. Interlobular fibrosis is well defined and more advanced than in most of the recovered sheep. $\times 24$.

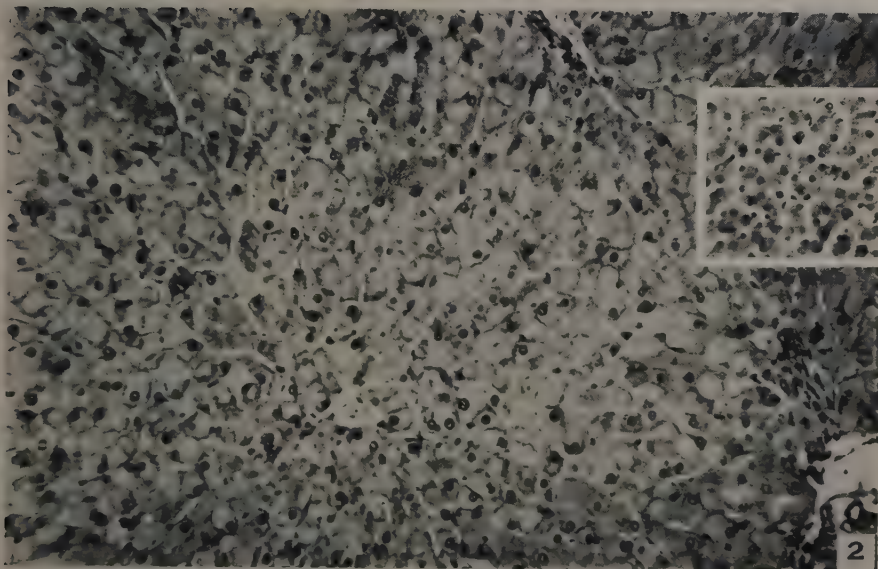
PLATE 4

Photograph of *Heliotropium europaeum*, well grown and flowering.

HELIOTROPE POISONING OF SHEEP

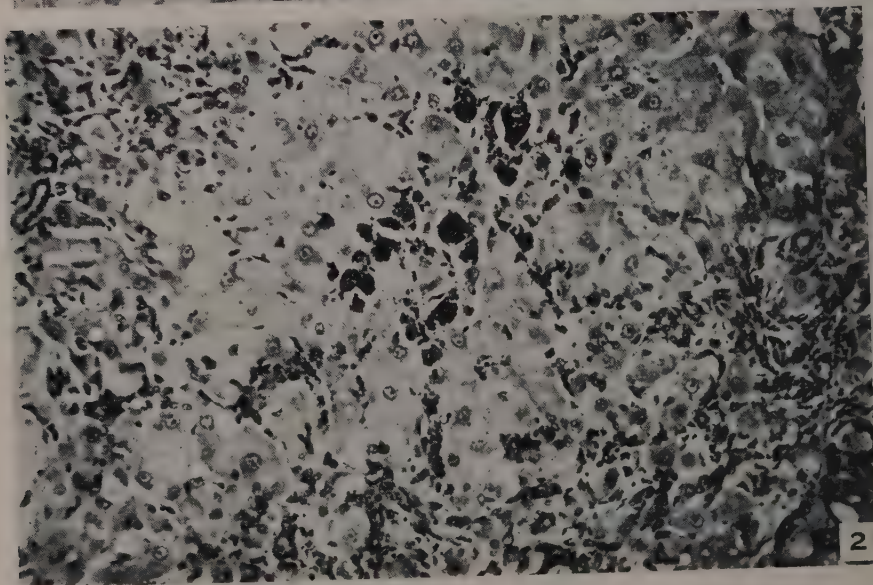
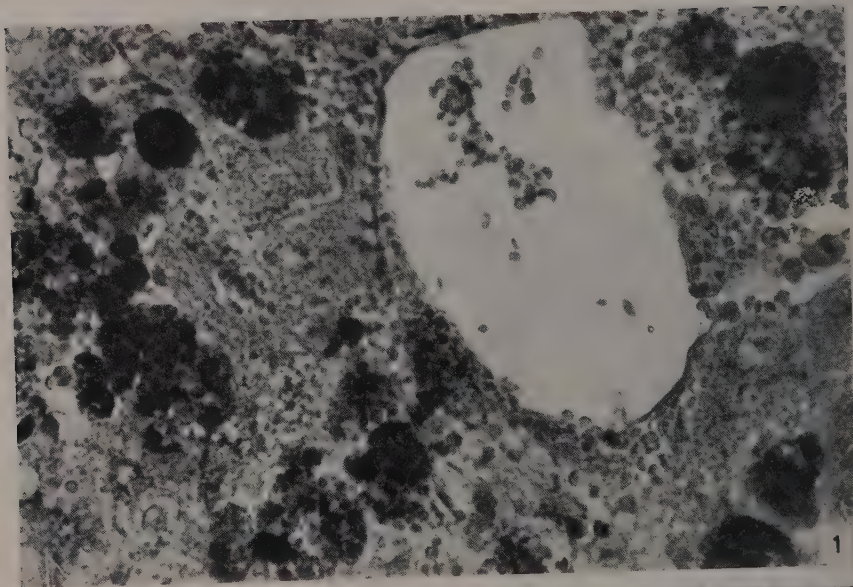


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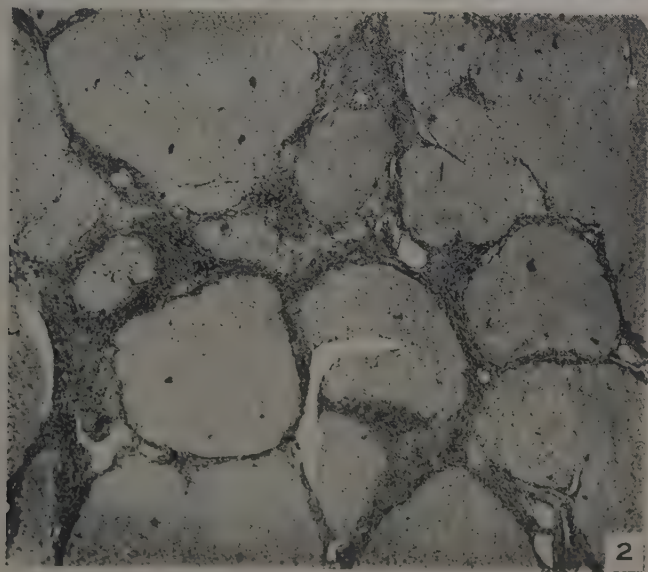
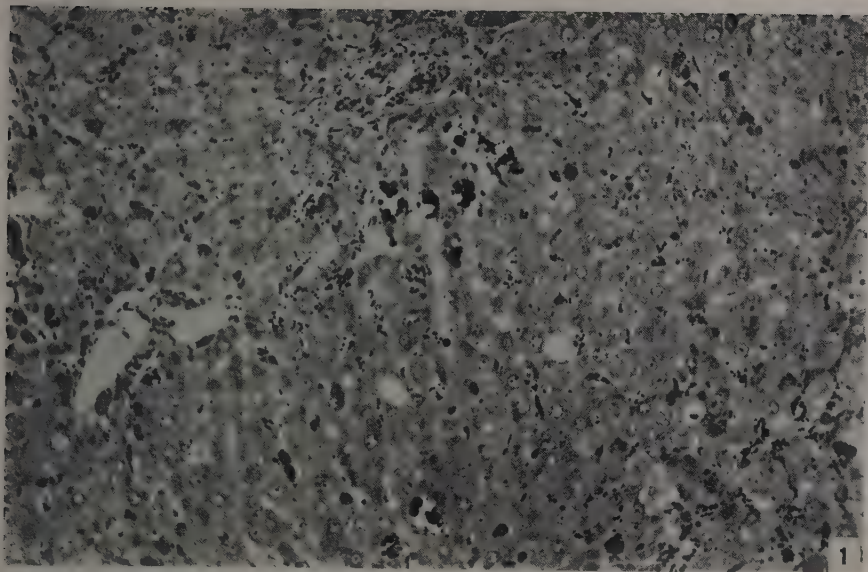


2

HELIOTROPE POISONING OF SHEEP



HELIOTROPE POISONING OF SHEEP



HELIOTROPE POISONING OF SHEEP



THE AETIOLOGY OF PHALARIS STAGGERS IN SHEEP

II. THE TOXICITY TO SHEEP OF THREE TYPES OF PASTURE CONTAINING *PHALARIS TUBEROSA*

By H. J. LEE,* R. E. KUCHEL,* and R. F. TROWBRIDGE*

(Manuscript received January 26, 1956)

Summary

The seasonal occurrence of phalaris staggers in sheep on three types of pasture containing *Phalaris tuberosa* L., during periods of 3 years, 2 years, and 1 year respectively, is described.

A relatively pure stand of phalaris proved toxic to sheep for almost 5 consecutive months of its growing period. The consumption of appreciable quantities of lucerne, subterranean clover, or other plants, along with the phalaris, reduced the hazard very considerably.

Neither the dormant tubers nor the standing straw of phalaris proved to be toxic.

The widespread occurrence of phalaris staggers on a diversity of soil types is reported and the possibilities that the toxicity of phalaris may fluctuate according to climate and terrain are discussed.

The view is advanced that actively growing phalaris is potentially toxic to sheep under most, if not all, circumstances.

I. INTRODUCTION

In the first paper in this series (Lee and Kuchel 1953a) it was shown that the onset of phalaris staggers, a malady in sheep that sometimes results from the consumption of the perennial grass *Phalaris tuberosa* L., could be prevented by the oral administration of cobalt chloride.

These experiments showed that in certain circumstances phalaris can be particularly toxic during the early stages of its seasonal growth; they left open the question as to whether phalaris continues to be toxic during the later stages of growth. Under field conditions the hazard decreases as the plant matures, but by this time the growth of other fodder species in the pastures is usually considerable and so the grazing animals consume less phalaris.

During the course of subsequent investigations, all of which were conducted near Keith in South Australia, within a mile of the site of the early experiment and on similar terrain, a considerable body of evidence has been gathered which bears on the toxicity of phalaris. This evidence is presented below.

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II. EXPERIMENTAL

(a) Pastures

Three separate pastures were employed, two of which were seeded with other species in addition to phalaris. The composition of the sown pastures, the time of their establishment, and the years in which they were grazed by experimental flocks are set out in Table 1.

All three pastures had been treated with copper and zinc as well as superphosphate at the time they were sown.

TABLE 1
THE EXPERIMENTAL PASTURES

Pasture	Species Established	Year of Sowing	Years Pastures Grazed Experimentally
1	<i>Phalaris tuberosa</i> Subterranean clover (<i>Trifolium subterraneum</i> L.) Lucerne (<i>Medicago sativa</i> L.)	1949	1951, 1952, 1953
2	<i>Phalaris tuberosa</i> Subterranean clover	1949	1952, 1953
3	<i>Phalaris tuberosa</i>	1952	1953

The sown species established well and constituted the bulk of the swards. In each instance a small proportion of extraneous species contributed in some measure to the total green feed available; most of these plants were annual grasses that were not prominent until they approached maturity in the spring. In an attempt to produce a pure stand of phalaris and to reduce this volunteer growth to a minimum, pasture 3 was developed in an area that had supported virgin scrub until this was destroyed immediately prior to the preparation of the seed bed.

(b) Sheep

Merino ewes and wethers, in several age groups, were used in all the relevant experiments. In this paper, consideration has been given only to those sheep that were susceptible to staggers because they were not protected by adequate cobalt supplements.

III. OBSERVATIONS

Many of the sheep that were confined to phalaris pastures scoured freely, irrespective of the experimental treatments they received. This scouring was evidently of nutritional origin, as rigorously applied anthelmintic measures failed to relieve the condition. It did not affect the course of the disease and has not been considered further.

The incidence of staggers on each of the pastures during the years 1951, 1952, and 1953, together with the conditions of climate and of plant growth that prevailed in these years, are discussed in detail below. It is on these considerations that the conclusions drawn have largely been based. These data are presented diagrammatically in Figure 1, in which the periods when the various species were growing and were present in quantities sufficient to be grazed are shown, but no attempt has been made to depict the relative abundance of these species in the swards.

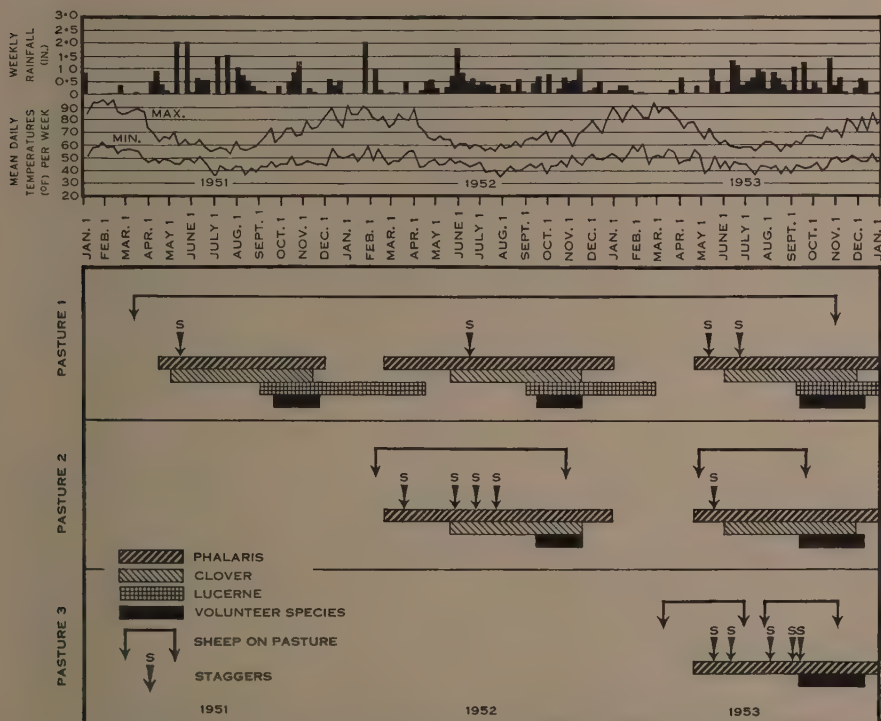


Fig. 1.—The incidence of fresh outbreaks of phalaris staggers during three consecutive years in sheep confined to experimental pastures near Keith, in South Australia. The periods when the various pasture components were growing and were estimated to be present in sufficient quantity to be grazed may be derived from the key.

(a) 1951 Season

The first three months of 1951 were hot and dry. The opening rains, falling early in April, favoured the immediate growth of phalaris and also the germination and subsequent growth of subterranean clover; they did not promote further growth in the already mature lucerne.

Mild weather with adequate rains ensured the uninterrupted growth of phalaris and of clover throughout the winter and spring; the clover

dried off in mid November, while the phalaris remained partially green throughout December.

Following the advent of warmer weather in September, the lucerne grew rapidly and contributed substantially to the sward throughout the remainder of the year.

(i) *Sheep on Pasture 1 (Phalaris, Clover, and Lucerne)*.—The appearance of staggers on this pasture provided the basis for the observations recorded by Lee and Kuchel (1953a).

Twenty-five ewes were depastured in this area in March 1951 when the pasture was dry. No untoward symptoms appeared in 10 ewes that were dosed with 7 mg cobalt each week, nor in their progeny. Amongst the 15 untreated ewes, however, symptoms of phalaris staggers were first noticed on May 14 and, by June 6, 11 of them were affected in varying degree; seven succumbed within a year. No further cases appeared amongst the surviving ewes after June 6.

In addition to these ewes, their progeny (born during April, May, and early June) remained with the flock until they were weaned in October; none of them developed staggers during this period. After weaning, these lambs, together with some 30 similar lambs from other pastures, grazed this area throughout the remainder of the year without ill effect.

(b) 1952 Season

In 1952, the first effective rain fell during the last week in January, when 2.06 in. was recorded. As a result of this rain, and a further fall of 0.96 in. 2 weeks later, lucerne grew luxuriantly, phalaris made appreciable growth, and a moderate germination of subterranean clover took place. Thereafter relatively hot, dry weather prevailed until the third week in March, when 0.49 in. of rain fell. In the meantime practically all of the newly germinated clover had died, the lucerne had ceased growing and had set seed, and the fresh growth of phalaris had begun to wilt. The March rain revived the phalaris somewhat but did little to establish the clover; the mature lucerne failed to make appreciable fresh growth.

During the latter half of April 1.33 in. was recorded, which ensured the continued growth of phalaris and brought about a fresh germination of clover.

With the advent of cold weather these new clover plants developed very slowly, despite adequate rains. Throughout the winter the growth of lucerne was negligible. As a result the winter feed (which was never abundant) consisted principally of phalaris until the clover made some further headway during August. In the spring all three species flourished.

(i) *Sheep on Pasture 1 (Phalaris, Clover, and Lucerne)*.—At the beginning of 1952 this area was grazed by the original ewes that had survived the 1951 outbreak of staggers, together with 40 hoggets. Of

these young sheep, 16 were removed to pasture 2 (see below) in February. The first symptoms of staggers were observed in some of the remaining hoggets in mid June and, when these sheep were removed early in July because the available green feed was insufficient to provide for them as well as for the ewes, seven of the 16 hoggets that were not dosed with cobalt exhibited unmistakable symptoms.

No fresh cases of staggers appeared in the ewes during 1952, nor were their lambs born during that year affected.

(ii) *Sheep on Pasture 2 (Phalaris and Clover)*.—Some of the experiments conducted on this pasture have been referred to briefly by Lee and Kuchel (1953b).

In early February, 16 hoggets were transferred from pasture 1 to part of this area, where they were inspected at weekly intervals. On March 17, approximately one-half of them showed early signs of staggers (i.e. 3 months before the comparable hoggets remaining on pasture 1 were affected). On April 4, a larger experimental flock of 60 hoggets was introduced to the same pasture; the first cases of staggers in this flock were detected on May 26 while fresh cases were recognized in mid June and again in mid July, after which time no more cases occurred. In all, 23 of the 60 animals were affected and of these, five had died by the time observations were discontinued in October.

(c) 1953 Season

During January 1953, isolated showers provided a total of 0.93 in. which, in the otherwise hot weather, sufficed only to promote a scanty growth of the perennial pasture species. The pastures remained dry throughout February and March, which were hot months. During the first week in April 0.68 in. of rain fell, and a further 0.40 in. was registered in scattered showers during the rest of the month. This rain induced a slow growth of phalaris but, as the weather was cooler, the lucerne responded little if at all. Subterranean clover germinated and persisted without, however, contributing materially to the bulk of the pastures.

The first two weeks in May were also dry but thereafter wet conditions prevailed throughout the winter and spring months. The cold and bleak winter was not favourable for the rapid growth of any species. With the advent of higher temperatures in mid September, both annual and perennial species grew vigorously.

(i) *Sheep on Pasture 1 (Phalaris, Clover, and Lucerne)*.—Of the 15 ewes that grazed this area at the beginning of 1953, eight had been protected by cobalt during the past 2 years.

During May, all seven of the untreated ewes and five of the eight ewes that had been treated with cobalt displayed the hyperexcitability associated with the initial appearance of staggers. In several of the untreated ewes these early manifestations were superimposed on chronic

symptoms that had persisted since the first occurrence of staggers in 1951. The disease progressed, and by the end of August all of the affected ewes were dead. The appearance of phalaris staggers in the cobalt-treated sheep was undoubtedly due to the greater toxic potential of this pasture in 1953 than during the previous 2 years and is referred to again in Section V.

These ewes had lambled in April. Because of the death or disability of their dams, their progeny had perforce to forage for sustenance at an unusually early age. Two of these lambs developed staggers when they were 2 months old. The surviving lambs were removed from the area in August, while the three remaining ewes grazed these pastures without ill effect until they were discarded in October.

On July 1, 78 1-year-old wethers were transferred to pasture 1 from pasture 3 (see below). At that time, early signs of staggers were apparent in several of these animals and obvious symptoms developed in these and many others during the ensuing 2 weeks. It is considered that the phalaris in pasture 3 precipitated the disease but it cannot be stated with certainty to what extent the two pastures were responsible for the ultimate condition of these sheep.

(ii) *Sheep on Pasture 2 (Phalaris and Clover).*—A flock of 200 mature wethers was transferred to this pasture on April 28. The first case of staggers in this flock was observed on May 19, and by the time the sheep were removed on June 4 approximately 10 per cent. were obviously affected. At least half of these died within a month.

A fresh flock of 80 similar wethers was introduced to this area on June 5 and remained there until the end of September. None of these sheep developed staggers.

(iii) *Sheep on Pasture 3 (Phalaris).*—After sowing phalaris on this area in July 1952, the plants were allowed to grow unchecked until December, when they were heavily grazed for 2 days at the flowering stage.

A small pilot flock of four sheep was introduced on March 10 to graze the small amount of green phalaris then available. One of these animals developed staggers by May 15, while two more were affected by June 6.

An experimental flock of 78 1-year-old wethers was transferred to the area on May 26 and, in an attempt to stimulate sufficient pasture growth to support these sheep, 2 cwt ammonium sulphate per acre was applied early in June.

Amongst these animals, the earliest signs of staggers were suspected on June 10. However, as the rate of growth of the phalaris proved insufficient to provide adequately for the flock, it became necessary to transfer them to pasture 1 on July 1. At that time, many of the animals were exhibiting the marked hyperexcitability of the premonitory phase of the staggers syndrome but the disease was not otherwise obvious.

Definite symptoms developed progressively in many of these sheep during the next 2 weeks.

The unaffected remainder of this flock was then regrouped for a further experiment and, on July 27, was returned to pasture 3, which by this time carried an ample body of phalaris. Amongst these sheep were 18 that had received protective doses of cobalt throughout the initial experiment; 12 of these had been dosed for the last time on July 17. By August 4, several of these 12 sheep were affected by staggers, and 1 week later 20 of the 26 animals exposed were staggering; 12 sheep that continued to be dosed with cobalt throughout this period remained unaffected. Six of these dosed animals were treated for the last time on August 11 and four were staggering by September 3. On that date, the final preventive dose of cobalt was administered to the remaining six unaffected sheep. One of these developed staggers by September 16 and was killed in the final stages of the disease 1 week later. All five of the remaining animals were visibly affected by the end of September.

TABLE 2
INCIDENCE OF STAGGERS ON EXPERIMENTAL PASTURES

Year	Pasture	Mar.	May	June	July	Aug.	Sept.
1951	1 (phalaris, clover, and lucerne)		×				
1952	1			×			
	2 (phalaris and clover)	×	×	×	×		
1953	1		×	×			
	2		×				
	3 (phalaris)		×	×		×	×

A further experiment with a fresh flock of sheep was started on September 14 and discontinued on November 4. During this interval, no symptoms of phalaris staggers developed in this flock.

The months in which fresh manifestations of staggers occurred on these experimental pastures during the three years under review are shown in Table 2.

(d) The Toxicity of Dormant Tubers and of Dry Straw

In January 1953, when the weather was hot and dry, clumps of dormant phalaris were ploughed up from pasture 2. When these were exposed the tubers dried rapidly. A considerable quantity of these was collected. The roots and stems were trimmed off and discarded; during this process the bulk of the adhering sandy soil was removed, and then the residual mats of tubers were subjected to vigorous treatment in a hammer mill that pulverized the remaining roots and stems while leaving the hard tubers almost intact. The finely divided plant material and the

remaining sand were screened off, leaving the tubers relatively free from contamination.

These tubers were then offered to two sheep in pens; they did not prove to be particularly palatable, but with the addition of a small amount of sucrose the tubers constituted the sole diet for these sheep for 30 consecutive days. Neither animal displayed any untoward symptoms.

Later, in mid January 1954, a quantity of dry phalaris straw was harvested from pasture 3. This material was chaffed and fed, with no alternative fodder, to one penned sheep for 3 weeks. The animal consumed between 800 and 900 g daily throughout this period without ill effect.

It is concluded, therefore, that the toxic principle present in green phalaris top growth is not stored in the tubers of the plant during dormancy and that it does not persist in the standing straw.

(e) *Guinea-pigs*

In an endeavour to determine the toxicity of phalaris to species other than ruminants, six adult male guinea-pigs were placed in a netted enclosure in pasture 3 on August 31, 1953. Water was provided and the only food available was phalaris, which they readily ate. From time to time, the enclosure was moved to provide fresh pasture.

These animals experienced bitterly cold weather soon after their arrival and, although a hutch was provided, three of them died within a fortnight, presumably from exposure. Two survived until observations were discontinued on November 4, 1953.

No untoward symptoms were observed in any of these guinea-pigs.

IV. ANALYTICAL

Forage samples were collected frequently throughout the experimental period. It seldom happened that lucerne, subterranean clover, phalaris, and the various extraneous pasture components were all present in any one pasture when the sampling was carried out, so specimens were taken not only from the experimental pastures but also from adjoining fields where they were to be found.

The concentration of cobalt in a representative selection of samples from these several sources, chosen to provide a comparison between as many species as were available in each instance, was determined by a modification of the method of Marston and Dewey (1940). The findings are set out in Table 3.

In these samples, lucerne invariably contained the greatest concentration of cobalt (the range encountered in the numerous lucerne samples that have been examined from this experimental site has been from 0.14 to 0.61 p.p.m. cobalt). The cobalt content of subterranean clover was frequently very low but, almost without exception, it was greater than that of phalaris collected at the same time.

In no instance did the volunteer species contain more than 0.07 p.p.m. cobalt and usually the cobalt concentration in these species did not exceed that in phalaris.

TABLE 3
THE CONCENTRATION OF COBALT IN PASTURE COMPONENTS
Results expressed as parts per million, dry basis

Species	4.vi.1951	1.viii.1951	10.x.1951	29.x.1952	5.xi.1953
Lucerne	0.28	0.28	0.36, 0.25	0.36	0.35
Subterranean clover	0.11, 0.08	0.12, 0.09	0.07, 0.05	0.05	0.08, <0.03, <0.02
<i>Phalaris tuberosa</i>	0.06	0.07	0.03	<0.02	0.09, 0.04, <0.02
<i>Hypochoeris</i> sp.					0.07
<i>Vulpia</i> sp.			0.02	0.02	0.05, 0.04, <0.02
<i>Lolium</i> sp.		0.04	0.07		
<i>Vulpia</i> sp. } *				0.07	
<i>Lolium</i> sp. }					
<i>Bromus</i> sp. } *					0.03
<i>Vulpia</i> sp. }					
<i>Bromus</i> sp. } *					<0.02
<i>Vulpia</i> sp. }					
<i>Lolium</i> sp. }					

* Composite sample.

V. DISCUSSION

In the non-irrigated pastures of southern Australia, the growth of phalaris is stimulated each year by late summer, autumn, or early winter rains after the plant has remained dormant during the greater part of the summer. Although most outbreaks of phalaris staggers occur during this initial period of growth, it is clear from the observations presented above that phalaris may remain toxic for a far greater proportion of its growing season; fresh outbreaks may occur under certain circumstances throughout the winter and early spring, provided the fodder consumed by the animals is predominantly phalaris.

Other pasture species growing in association with phalaris have a profound influence on the toxic hazard. The staggers syndrome was not observed at any time in flocks confined to phalaris pastures that contained an appreciable proportion of lucerne, for this palatable plant was readily consumed by the sheep at all stages of its growth. The late summer rains that promoted the growth of both phalaris and lucerne in February 1952 resulted in the occurrence of staggers in March on pasture 2 (phalaris and

clover) but not on pasture 1 (phalaris, clover, and lucerne) where the lucerne as well as the phalaris flourished. Sheep were affected on both of these pastures, however, in June after the growth of lucerne had been negligible for several weeks.

On the other hand, close observation of the grazing habits of the sheep left no doubt that they selectively grazed the phalaris and avoided the subterranean clover when these two species grew together early in the season. The relative unpalatability of the young clover would appear to account for the appearance of staggers on pasture 1 and pasture 2 in the late autumn or early winter of each of the three years under review. However, when the more mature clover was consumed in appreciable quantities as each season advanced, the grazing animals did not develop the staggers syndrome.

During the winter of 1953, the contribution of species other than phalaris to the sward in pasture 3 was negligible; it consisted mainly of the leaves of a few mallee (*Eucalyptus* sp.) shoots. Fresh outbreaks of staggers developed under these conditions until early in the spring. With the advent of warmer weather in September, several hitherto inconspicuous species (principally the annual grasses *Vulpia*, *Lolium*, *Bromus*, and *Hordeum* spp., together with the herbaceous plant *Hypochoeris radicata* L.) grew vigorously and, both from direct observation of grazing behaviour and from close inspection of the grazed sward, it became apparent that these plants were eagerly sought and consumed by the sheep. Although the phalaris continued to grow luxuriantly, no further cases of staggers appeared after the sheep were able, by selection, to consume a relatively high proportion of these volunteer species.

This moderating effect of other species on the occurrence of phalaris staggers was so marked that it seems likely that in these pastures freshly growing phalaris is toxic at all times, and that the staggers syndrome is precipitated only when the phalaris is consumed almost exclusively, either from choice or because of the lack of alternative grazing.

Consideration of the analytical data suggests that the protection afforded by other plants consumed in conjunction with phalaris could be due either to the additional cobalt some of them (lucerne in particular) provide or merely to the dilution of the toxic material present in phalaris to a level at which it can be metabolized safely by the sheep. Both of these mechanisms could operate simultaneously. The observations, however, suggest that the second alternative is more likely to be generally correct, as more often than not the protective species contain no greater concentration of cobalt than phalaris itself.

In 1953, the appearance of phalaris staggers on pasture 1 in sheep that were dosed with 7 mg cobalt each week — a regimen that had protected them for the previous 2 years — suggests that the phalaris was more toxic in 1953 or, alternatively, that phalaris constituted a greater propor-

tion of their diet than previously. The first alternative is supported by our observations (unpublished data) that larger (or more frequent) supplements *invariably* prevent staggers; and by other collateral evidence.

It is probable that the toxicity of *Phalaris tuberosa* fluctuates according to its stage of growth and to the terrain on which it is grown, and it may be inferred that under some circumstances phalaris may not contain sufficient toxic material to be harmful. Undoubtedly there are phalaris pastures in Australia and elsewhere which, because of their low toxic potential or because of the protection afforded by other forage plants growing in association, have never induced the toxic syndrome in sheep, but in the present state of knowledge there is no certainty as to the primary reason for this. It is clear, however, that on certain types of terrain (of which the experimental areas discussed here are typical) the toxic principle present in *Phalaris tuberosa* that is responsible for the staggers syndrome is present in the leaves of the plant during practically the whole of their growing period. The hazard of the malady is thus seemingly always potential; it is greatest when phalaris overwhelmingly dominates the pasture and especially when, after the first rains, the fresh young shoots comprise the only green pasturage for the grazing flocks.

The experimental findings presented above refer specifically to a restricted locality where the variations in climate and soil were negligible in the three closely adjacent pastures, but it is probable that their significance has a much wider application.

There is ample evidence that, under circumstances that involve a diversity of soil and climatic factors, *Phalaris tuberosa* is toxic to sheep, and also to cattle, in many widely separated localities in New South Wales (Bull, Edgar, Fearn, Johnstone, Roe, and Southcott, personal communications; McClymont 1954), in Victoria (Hewitt and Vasey, personal communications), the Australian Capital Territory (Willoughby, personal communication), South Australia (McDonald 1942; Lee and Kuchel 1953*a*, 1953*b*; Smith 1953; Symons 1953), Western Australia (Watson, personal communication), and also in New Zealand (Le Soeuf 1948; Iverson and McLean, personal communications).

It seems likely, therefore, that actively growing phalaris may elaborate a toxic constituent under most circumstances.

VI. ACKNOWLEDGMENTS

The unstinted cooperation of the Australian Mutual Provident Society, on whose property the experimental pastures were established, has made these investigations possible. The authors' grateful thanks are due also to Mr. J. K. Powrie of the Department of Agriculture, South Australia, for the identification of the forage plants; and to their Divisional colleagues Messrs. B. F. Good and A. M. Snoswell, who carried out the cobalt analyses, and Mr. D. W. Dewey who supervised these analyses.

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ADDENDUM

Since this paper was submitted for publication, a communication (Milne 1955) has been seen that describes an occurrence of phalaris staggers in sheep near Dunedin in New Zealand. The outbreak occurred in a flock that was confined to the pasture (phalaris, browntop, white clover, and red clover) in October when, because of unusual seasonal conditions, phalaris predominated. This observation supports the view that phalaris may be potentially toxic throughout the growing season.

FAT LAMB STUDIES IN VICTORIA

I. AN ASSESSMENT OF THE RELATIVE VALUE OF VARIOUS EXTERNAL MEASUREMENTS FOR DIFFERENTIATING BETWEEN VARIOUS GRADES OF EXPORT LAMB CARCASSES

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(Manuscript received November 15, 1955)

Summary

Sixteen measurements were made on 240 intact export lamb carcasses, representing 12 weight-grade classifications of 20 each, with the object of determining measurements likely to be of value for the assessment of conformation. Six were immediately discarded as being of little value.

The remaining 10, which included three probe measurements, were subjected to statistical analysis to determine linear combinations of measurements most convenient for discriminating between grades in each of three weight categories, namely, light (27-28 lb), medium (35-36 lb), and heavy (43-44 lb).

The 10 measurements were length of leg (*F*), depth of thorax (*Th*), thickness of loin (*BC*), thickness of flank (*P*), length of carcass (*K*), thickness of shoulder (*Sh*), width of shoulder (*W*), width of flank (*E*), width of gigots (*G*), and twist (*Z*), with discriminating capacity roughly in that order.

BC, *P*, and *Sh* were all estimates of muscle and fat obtained by using a probe, and results obtained compared favourably with overseas measurements made on the cut carcass. *E*, *G*, and *Z* had little or no discriminating capacity. Optimum discriminations between grades were given within each weight category by the following combinations of measurements: light—*F*, *K*, *BC*, *Sh*, *P*; medium—*F*, *Th*; heavy—*F*, *K*, *W*, *P*; overall—*F*, *Th*, *BC*, *P*.

The groups of carcasses separated into clear weight and grade zones, and several trends were noticeable. The most important of these were: (i) grading of light-weight carcasses was highly subjective between all grades, as both conformation and fat cover were considered; (ii) grading of the top grades in heavier carcasses was more clear-cut and depended primarily on conformation; (iii) there was a tendency for heavier carcasses to be up-graded relative to light carcasses, owing to their heavier fat cover.

In view of the fairly clear pattern that emerged from the statistical model used, it is suggested that a rounded form of a discriminant function, namely,

$$Y' = 0.057F + 0.050Th - 0.097BC - 0.17P,$$

might be a useful index of conformation for fat lamb carcasses of all weights particularly if used in the form:

$$\text{Conformation score} = 250 - 10Y'.$$

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I. INTRODUCTION

Hammond (1952) considers the best definition of carcass quality is that which the public likes best, and grading of lamb carcasses is based on this concept. Light-weight, "blocky" carcasses, with a good fat covering, make the highest grades and command the greatest price per pound.

Grading of lambs for export is subjective, and in view of the numbers of carcasses handled must always be so. However, for competitions and for experimental purposes an objective approach should be taken, and a quantitative assessment be made of the value of the carcass in terms of both "quality" and composition.

This presents great difficulties. Firstly, lamb carcasses are not normally cut prior to export. Although there are two systems of appraisal based on a points system, namely, the Smithfield block test (Australian Meat Board 1952) and that of Hammond (1952), each involves cutting the carcass. This precludes their use on lambs appraised in Australia before export. Secondly, the value given to individual measurements is rather arbitrary and the difficulties in correlating them with actual carcass composition are enormous, involving detailed carcass dissections.

Pálsson (1939), Walker and McMeekan (1944), and Clarke and McMeekan (1952) have each tackled this task and have attempted to correlate external and internal carcass measurements with carcass composition as determined by dissection. All agree that (length of tibia + tarsus) is a good index of bone development. Pálsson considers (length of leg - length of tibia + tarsus) to be the best index of muscle development, but Walker and McMeekan consider the length of leg to be adequate alone. Coop and Clark (1952) appear to be of the opinion that length of leg is the best single index of carcass "quality". All are agreed that measurements made on the cut face of the carcass, cut at the last rib, are of great value. Depth of eye muscle and thickness of fat over the eye muscle and over the flank are particularly useful measurements. It is impossible to make direct measurements of these characters without cutting the carcass, but it is possible to make estimates of the thickness of fat plus muscle by use of a probe.

This paper reports results of an investigation conducted to determine the relative value of a number of external and probe measurements for the purpose of differentiating between commercial grades of fat lamb carcasses. Measurements were made during the lamb-killing season, October to December 1952, at the Brooklyn (Vic.) slaughter works of Thos. Borthwick & Sons Ltd.

II. MATERIALS AND METHODS

(a) *Grading System*

The system of grading used by Borthwicks conforms to the Victorian standard and is shown in Table 1. The system is two-dimensional, and

is based on conformation, signified by a trade name, and on weight, signified by a letter or numeral. No direct account is taken of breed, but obviously the breed or cross will be reflected in the grade. The two top grades, "Down Royal" and "Royal", each represent good quality carcasses well covered with fat, and differ only in conformation. Down Royal (commonly referred to simply as "Down" grade) has the characteristic short bones and "blocky" conformation associated with the Down breeds of sheep. Royal grade carcasses appear rather longer in the leg and body. The two lower grades ("Tallarook" and "Fir") show progressively less fat cover, and this appears to be the main grading criterion.

TABLE 1
COMMERCIAL GRADES OF FAT LAMB CARCASSES
System used by Thos. Borthwick & Sons Ltd.

Weight Range (lb)	Grade				
	Down	Royal	Royal	Tallarook	Fir
Up to 28	D		D	D	L
29 to 36	2		2	2	O
37 to 42	8		8	8	
43 to 50	4		4	4	
51 to 56			T		

Heavy carcasses tend to have a greater fat cover than light ones of comparable shape, and so tend to be graded up. Thus most carcasses of over 40 lb have sufficient fat cover to grade Tallarook or better, while those over 50 lb all make Royal grade. It should be noted, however, that very heavy carcasses are discriminated against in top quality grading. Carcasses of over 50 lb weight are not graded Down Royal even though they may be of Down type, but are lumped together in Royal grade. This results in a wider diversity of conformation in this weight-grade classification than in others.

(b) *Method of Sampling*

Following a number of preliminary measurements on some 50 carcasses of various weights and grades, a systematic study was made on 240 carcasses representing 20 in each of 12 weight and grade classifications. Four weight categories each covering a range of 2 lb and separated by intervals of 8 lb were chosen, and 20 carcasses measured in each for all the grades of quality available (Table 2). Weights were classed as light (27-28 lb), medium (35-36 lb), heavy (43-44 lb), and very heavy (51-52 lb).

The daily target was 20 carcasses. In order to reduce sampling errors the daily tally included no more than five of any one weight-grade classification.

(c) *Measurements*

Sixteen measurements were made on each uncut carcass. Of these, six were discarded for various reasons. These were: (1) length of fore-

TABLE 2
FAT LAMB CARCASS MEASUREMENTS: NUMBER OF CARCASSES MEASURED IN EACH
WEIGHT-GRADE CLASSIFICATION

Grade	Weight Category			
	Light (27-28 lb)	Medium (35-36 lb)	Heavy (43-44 lb)	Very Heavy (51-52 lb)
Down Royal	20	20	20	N.A.*
Royal	20	20	20	20
Tallarook	20	20	20	N.A.
Fir	20	20	N.A.	N.A.

* N.A., none available.

quarter, (2) length of loin, (3) and (4) fullness of thighs and depths of gigots (*I* and *H* of Pálsson 1939), (5) circumference of ham, and (6)

TABLE 3
DESCRIPTION OF MEASUREMENTS ARRANGED IN ORDER OF RELATIVE DISCRIMINATING VALUE

Code	Description	Ease of Measurement	Discriminating Value	Relation to Measurement Described by Pálsson (1939)
<i>F</i>	Length of leg	Easy linear	Very good	As described
<i>Th</i>	Depth of thorax	Easy calliper	Good	As described
<i>BC</i>	Thickness of loin	Fairly easy probe	Good	Thickness of eye muscle (<i>B</i>) + fat cover (<i>C</i>)
<i>P</i>	Thickness of flank	Easy probe	Good	Thickness of muscle on lower half of rib (<i>X</i>) + fat cover (<i>Y</i>)
<i>K</i>	Length of carcass	Easy linear	Fairly good	As described, but measured from crutch rather than base of tail
<i>Sh</i>	Thickness of shoulder	Difficult probe	Fair	New measurement
<i>W</i>	Width of shoulder	Easy calliper	Fair	New measurement
<i>E</i>	Width of flank	Easy calliper	Little value	New measurement
<i>G</i>	Width of gigots	Easy calliper	No value	As described
<i>Z</i>	Twist	Difficult circular	No value	New measurement

circumference of loin. Measurements (1), (2), and (3) were difficult to make accurately, while (4), (5), and (6) appeared to be of no value for purposes of discrimination between grades.

The remaining 10 measurements are defined and illustrated in Table 3 and in Figure 1. Where similar measurements have been made the system of nomenclature is similar to that used by Pálsson (1939). No further description is necessary for measurements *F*, *Th*, *K*, and *G*. The remaining six need some amplification.

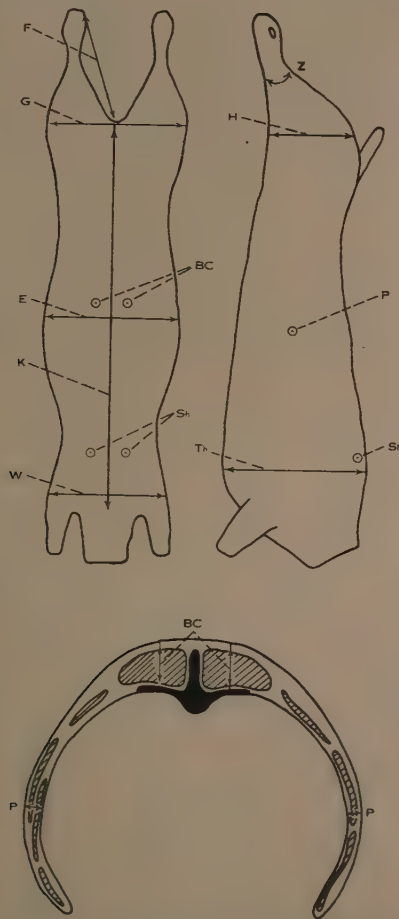


Fig. 1.—Diagrammatic representation of the 10 measurements used in the analysis.

The thickness of loin (*BC*) was measured by passing a skewer right through the carcass from a point offset from the midline of the back a distance approximately 10 per cent. of leg length (*F*), and immediately above the transverse process of the first lumbar vertebra. This was in order to locate accurately the position of the skewer relative to the appropriate vertebra. The point was then withdrawn and taken to the

flattened area of the transverse process. The position of the surface of the carcass was marked on the skewer which was then withdrawn and the depth of penetration measured. A similar technique was used to measure the thickness of shoulder (*Sh*) except that the point was offset from the midline by approximately 15 per cent. of *F*, and the measurement was made to the seventh rib. Thickness of flank (*P*) was measured midway, dorsoventrally, between the second and third last ribs. The skewer was passed right through, then withdrawn till the point barely penetrated, and the depth recorded. The width of shoulder (*W*) and of flank (*E*) were made with callipers across the widest parts. The twist (*Z*) was measured by passing two skewers at right angles through the leg muscle on a plane with the patella. A string was passed around below the skewers and measured.

Where applicable, measurements of *F*, *BC*, *P*, *Sh*, and *Z* were made on each side of the carcass and the mean recorded. All measurements were made a few hours after slaughter and prior to chilling. Carcass weights cited are estimated frozen weights, i.e. fresh weights minus 4 per cent.

(d) *Analysis of Data*

The data were dealt with in two ways. Firstly, they were examined in a general way to determine weight-grade-measurement relationships for a comparison with results of similar studies made overseas.

Secondly, two generalized discriminant analyses of the measurements were carried out. This statistical technique is an extension of Fisher's well-known method of constructing a discriminant function for two groups (see Rao 1952). It consists of finding certain linear combinations (i.e. weighted sums, both positive and negative weights being admitted) of the observations. These linear combinations are constructed so as to have the following properties: the first discriminant function is that linear combination which maximizes the ratio of its sum of squares between groups to its sum of squares within groups. The second combination is chosen from the class of combinations which appear to be uncorrelated, within groups, with the first; from this class, the one is chosen which maximizes a ratio analogous to that used in determining the first discriminant function. The third combination is uncorrelated with the first two and maximizes a similar ratio, and so on. Descriptions of the computational processes yielding these combinations, and references to the tests of significance employed to decide how many combinations should be formed, have been published (e.g. Binet and Watson 1956).

In the first generalized discriminant analysis, the 12 weight-grade classifications were regarded as groups. In this analysis three significant components were found. For demonstration purposes these three components may be used to define a set of three mutually perpendicular axes. The location of the mean point for each of the 12 weight-grade classifica-

TABLE 4
ROUNDED MEAN VALUES FOR MEASUREMENTS SHOWN IN TABLE 3

Weight Category	Grade	Measurements in Order of Discriminating Value (mm)									
		<i>F</i>	<i>Th</i>	<i>BC</i>	<i>P</i>	<i>K</i>	<i>Sh</i>	<i>W</i>	<i>E</i>	<i>G</i>	$10\sqrt{Z}$
Light (27-28 lb)	Down	213 (199-223) *	238	31	14	706	42	171	205	200	152
	Royal	228 (216-251)	241	29	12	715	41	168	201	199	152
	Tallarook	252 (224-273)	252	27	10	742	42	163	198	200	151
	Fir	264 (244-283)	258	24	9	764	36	160	195	202	151
Medium (35-36 lb)	Down	223 (211-241)	255	33	16	763	46	189	222	211	157
	Royal	257 (240-278)	262	32	15	783	48	181	218	211	156
	Tallarook	263 (240-280)	273	30	12	802	43	178	219	212	156
	Fir	277 (265-297)	277	28	10	815	41	176	215	213	156
Heavy (43-44 lb)	Down	232 (213-247)	270	37	20	815	49	208	236	219	162
	Royal	262 (247-285)	280	34	16	830	48	196	236	221	160
	Tallarook	268 (243-297)	285	32	15	847	48	196	232	221	162
Very heavy (51-52 lb)	Royal	250 (224-286)	284	38	22	855	53	212	253	225	166
Pooled standard deviation		11.3	7.3	2.1	2.0	17.0	4.4	7.2	10.5	4.9	2.6

* Figures in brackets indicate range. These are presented only for *F*, as *F* is the most significant measurement.

tions can then be determined relative to the axes. This three-dimensional model is illustrated in Figure 2. Approximately 71 per cent. of the variability between the means for the 12 classifications is accounted for by differences mainly due to carcass weight (*X*-dimension). Differences mainly due to grade account for about 95 per cent. of the remaining variability between means (*Y*-dimension). Almost all the variability remaining is taken up by the component in the *S*-dimension. The coefficients appearing in these weighted sums were adjusted so that the variance of any score calculated for a single carcass has an estimated value of unity.

TABLE 5
TABLE OF GROSS CORRELATION COEFFICIENTS
Pooled within all weight-grade classifications

Measure- ment	<i>F</i>	<i>Th</i>	<i>BC</i>	<i>P</i>	<i>K</i>	<i>Sh</i>	<i>W</i>	<i>E</i>	<i>G</i>
10√ <i>Z</i>	-0.257	-0.003	0.115	-0.192	0.033	0.147	0.081	-0.112	0.386
<i>G</i>	-0.094	0.095	0.041	-0.230	-0.007	0.041	0.072	0.111	
<i>E</i>	-0.252	-0.138	0.138	0.138	-0.271	0.058	0.255		
<i>W</i>	-0.186	-0.250	-0.072	0.180	-0.072	0.038			
<i>Sh</i>	-0.045	-0.053	0.219	0.038	-0.132				
<i>K</i>	0.400	0.266	-0.356	-0.189					
<i>P</i>	-0.142	-0.117	0.024						
<i>BC</i>	-0.260	-0.153							
<i>Th</i>	0.244								

In addition to the overall discrimination between the 12 weight-grade classifications, scores were calculated which would give maximum discrimination between grades within each weight category. This was achieved by a second discriminant analysis in which each weight category was treated separately, and the different grades of the same weight were the groups between which discrimination was required. In the scores so obtained, the differences between grades in the cumulative effects of the measurements used accounted for about 93 per cent. of the total variation. This component was denoted by *U*. The remainder of the variability in the measurements was due to apparent inconsistencies in some of them. The component which took up practically all of this variation was denoted by *V*. These results are illustrated in Figures 3, 4, and 5 for light, medium, and heavy carcasses respectively.

III. RESULTS

(a) General Considerations

Table 4 presents the means of the 10 measurements. For convenience of statistical analysis the twist measurement (*Z*) is expressed as 10√*Z*. Table 5 shows the gross correlation coefficients between measurements pooled within all weight-grade classifications.

TABLE 6
RELATIVE VALUES OF MEASUREMENTS EXPRESSED AS PROPORTIONS OF VALUES FOR LIGHT-WEIGHT DOWNS

Weight Category	Grade	Relative Weight*	Relative Measurement†							
			F	Th	BC	P	K	Sh	W	10√Z
Light (27-28 lb)	Down	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Royal	1.00	1.07	1.01	0.93	0.82	1.01	0.97	0.98	1.00
	Tallarook	1.00	1.18	1.06	0.87	0.72	1.05	1.00	0.95	0.99
	Fir	1.00	1.24	1.08	0.77	0.64	1.08	0.87	0.94	0.99
Medium (35-36 lb)	Down	0.78	0.82	0.83	0.83	0.88	0.84	0.86	0.86	0.80
	Royal	0.78	0.94	0.86	0.81	0.81	0.86	0.89	0.82	0.80
	Tallarook	0.78	0.96	0.89	0.75	0.67	0.88	0.80	0.81	0.80
	Fir	0.78	1.01	0.91	0.70	0.57	0.90	0.76	0.80	0.80
Heavy (43-44 lb)	Down	0.64	0.69	0.72	0.75	0.88	0.73	0.74	0.77	0.68
	Royal	0.64	0.78	0.75	0.70	0.72	0.75	0.73	0.73	0.67
	Tallarook	0.64	0.80	0.76	0.66	0.66	0.76	0.73	0.73	0.67
	Royal	0.54	0.63	0.64	0.66	0.84	0.65	0.63	0.67	0.58

* Relative weight = 28/wt.

† Relative measurement = measurement/wt.

By plotting the mean measurements for all grades in each weight category against carcass weight it can be shown that the lines for W , $10\sqrt{Z}$, G , E , K , and Th do not deviate significantly from linearity. All these measurements continue to increase at a more or less uniform rate over the weight range represented by carcasses examined in this study. Measurements F , Sh , and BC tend to increase at a decreasing rate, suggesting them to be relatively early-maturing. Measurement P shows a tendency to increase at an increasing rate, suggesting it to be late-maturing, undoubtedly owing to the large contribution made to this measurement by fat deposition.

Relative changes in measurements can be demonstrated by dividing them by carcass weight and expressing the values so obtained as a proportion of those obtained for a standard weight-grade classification (Table 6). Within each weight category and with decrease in grade, F increases at the greatest rate, followed by Th and K . No trends are shown by G , E , or Z . Measurement P decreases at the greatest rate, followed by BC , Sh , and W .

(b) *Multivariate Analysis*

(i) *All Carcasses*.—The four measurements F , Th , BC , and P were chosen for overall discrimination on the basis of their roles in discrimination within weight categories. Three significant components were extracted, the values for which are presented in Table 7 and illustrated as a three-dimensional model in Figure 2.

The weighted sum X —yielding maximum overall discrimination—accounts for 71 per cent. of the total variability between groups in these measurements. It is apparent that differences in the X -dimension are due primarily to differentiation into clear-cut weight zones, representing the *absolute* increase in all four measurements with increase in weight.

It may be noted, however, that the point LD (light-weight Downs) is out of line with the remainder of the light-weight carcasses, owing to a slightly high relative value of Th .

The second principal component, Y , takes up 95 per cent. of the remaining variability. Differences in this dimension seem to be due to differentiation into grades, which is a result of a *relative* decrease in length of leg and depth of chest, and a *relative* increase in thickness of loin and of flank, from the lowest to the highest grade.

In this dimension four tendencies are apparent. Firstly, the separation of the Down grade from the rest is smaller in the light-weight carcasses than in the other weight categories. Secondly, there is a better discrimination between the Down and Royal grades of medium- and heavy-weight carcasses than between any other adjacent grades at these weights. Thirdly, placement of corresponding grades, between weights, is skew.

With each increase in weight every grade, with the exception of the very heavy Royal, is displaced to the right. That is, it has a larger value for Y . Fourthly, the group representing the very heavy Royal grade (51-52 lb) does not conform to this pattern, and lies between the positions which one

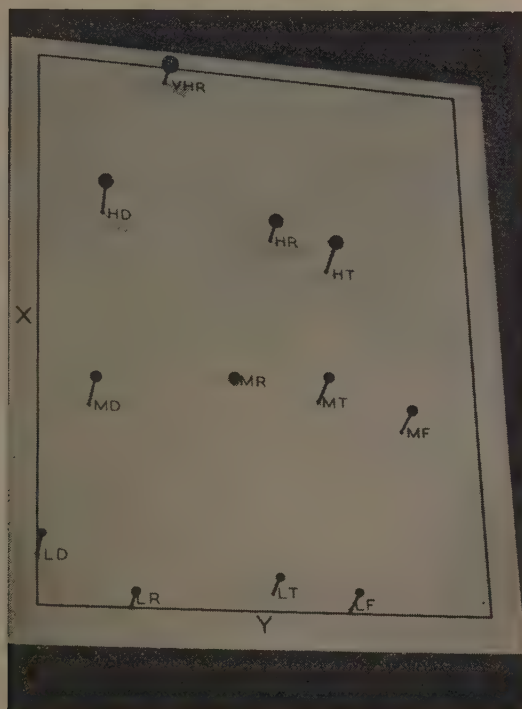


Fig. 2.—Three-dimensional model showing discrimination into weight and grade zones using the four measurements: length of leg (F), depth of thorax (Th), thickness of loin (BC), and of flank (P) (refer to Table 7).

$$X = 0.0182F + 0.0903Th + 0.2954BC + 0.3012P$$

$$Y = 0.0567F + 0.0501Th - 0.0968BC - 0.1688P$$

$$S = -0.0733F + 0.0978Th - 0.1346BC - 0.1464P$$

X is largely accounted for by differences in weight and Y by grade, while S (vertical dimension) is largely unaccounted for.

L, light (27-28 lb); M, medium (35-36 lb); H, heavy (43-44 lb); VH, very heavy (51-52 lb). D, Down grade; R, Royal; T, Tallarook; F, Fir.

would expect to be occupied by Down and Royal grades if both were available.

In the XY -plane, the medium and heavy categories form slightly convex curves; this is largely due to a relatively high value of X for the Royal grade samples, as a result of the relatively long legs (F) of these carcasses.

The *S*-dimension accounts for 70 per cent. of the remaining $1\frac{1}{2}$ per cent. of the overall variability and is almost certainly due to elements similar to those discussed subsequently in connexion with the analyses within weight categories. Two tendencies are apparent: (1) the points for light-weight carcasses lie close to a plane; (2) the points for Royal grade medium and heavy carcasses are considerably displaced. It seems that, but for the very discrepant *S*-value of medium-weight Royal carcasses, no significant third principal component would have appeared.

TABLE 7

MEAN VALUES OF THE THREE SIGNIFICANT COMPONENTS, *X*, *Y*, AND *S*, FOR OVERALL DISCRIMINATION BETWEEN WEIGHT-GRADE CLASSIFICATIONS OF CARCASSES
Formulae for *X*, *Y*, and *S* are given in Figure 2

Weight Category	Grade	<i>X</i>		<i>Y</i>		<i>S</i>	
Light (27-28 lb)	Down	38.8		18.6		1.41	
	Royal	37.9		20.2		1.25	
	Tallarook	38.3		22.6		1.19	
	Fir	38.0		24.0		1.36	
Medium (35-36 lb)	Down	41.6		19.5		1.73	
	Royal	42.3		22.1		0.27	
	Tallarook	41.9		23.6		1.63	
	Fir	41.4		25.1		1.51	
Heavy (43-44 lb)	Down	45.4		19.8		1.63	
	Royal	45.1		22.8		1.20	
	Tallarook	44.6		23.8		1.72	
	Fir	—		—		—	
Very heavy (51-52 lb)	Royal	48.2		21.0		1.09	
		—		—		—	

Undoubtedly sampling and measurement errors contribute to the apparent inconsistencies in the form of this model. None the less the greater part can readily be explained on the basis of the system of grading, as practised.

Any carcass which is known with certainty to fall into one of the 12 weight-grade classifications can be placed by calculating its *X*-, *Y*-, and *S*-scores from the formulae given, then putting it into that category to the mean scores of which these scores are nearest. The distance between two scores is defined as

$$\sqrt{\{(X_1 - X_2)^2 + (Y_1 - Y_2)^2 + (S_1 - S_2)^2\}}.$$

If one could safely exclude the possibility that the carcass in question belonged to the medium-weight Royal grade classification, then its *S*-score could well be ignored. An analogous process using the appropriate formulae

leads to rather more accurate results with less computation, if it is known — as in practice it usually is — to which weight category the carcass belongs.

Table 8 presents estimated expected misclassification rates between carcasses of all weights and grades. The discrimination between top and bottom grades within each weight category is uniformly good. There would be a probability of misclassifying only 2.1 to 2.2 per cent. of carcasses of all weights between the Down and Tallarook grades, if classification depended entirely on application of X and Y as given with Figure 2, using the four measurements F , Th , BC , and P . However, the chances of misclassification between adjacent grades are quite high. Of particular interest are those between Down and Royal grades in which the chances of misclassification decrease from 18.4 to 9.7 to 6.8 per cent. for light-, medium-, and heavy-weight carcasses respectively. Correspondingly, the chances of misclassification between Royal and Tallarook grades progressively increase from 11.1 to 28.4 per cent. with increase in weight.

The additional inclusion of S in the classification does not appreciably reduce the estimated expected misclassification rates, except when applied to the medium-weight Royal grade carcasses, in which it yields decreases of the order of 10 to 30 per cent. None the less, in view of difficulties involved in interpreting the expression representing S , and the very small fraction of the total variability contained in it, these reduced rates must be regarded with some caution and are not presented in detail.

(ii) *Light-weight Carcasses* (27-28 lb).—Significant improvement in discrimination is obtained at each instance by the successive inclusion of each of the following five measurements: F , K , BC , Sh , and P (Fig. 3), of which two are linear and three are probes.

The expression for U shows the relative importance, for quality grading at these weights, of a decrease in length of leg and of body, and of an increase in thickness of fleshing on the loin, flank, and shoulder.

The expression for V represents the departure from uniformity of the five measurements as constituting a series between grades. It seems that if each measurement formed a uniformly graded series and if there were no sampling and measurement errors then no significant component would appear in the dimension of V ; that is, practically all the difference between the four samples of 20 carcasses could be accounted for by differences in commercial grade. The values for V appear to depend non-linearly on those of U , for which two trends in the measurements are responsible. Firstly, the decline in the value of V from Down to Tallarook grade is mainly due to excessive decrease in the value of P . In view of the extremely thin flanks at this weight this could be due to errors of measurements. Secondly, the increase in V from Tallarook to Fir grade is due mainly to a disproportionate decrease in the value of Sh . The shoulder is an early-maturing part of an animal (Hammond 1932) and this can be seen from

our data (Table 4). Light-weight animals of Tallarook grade have a thickness of shoulder equal to that of Down grade, despite markedly thinner flanks and loin. Between Tallarook and Fir grades a distinct break appears; the shoulders are relatively immature in the lowest grade.

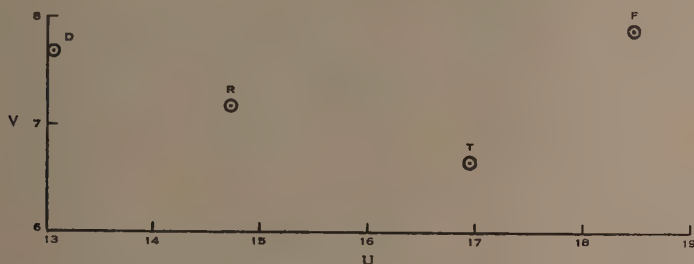


Fig. 3.—Maximum discrimination between grades of light-weight carcasses (27-28 lb) obtained using functions of measurements F , K , BC , Sh , and P .

$$U = 0.0552F + 0.0123K - 0.1513BC - 0.0092Sh - 0.1619P,$$

$$V = -0.0271F + 0.0263K - 0.0508BC - 0.1650Sh + 0.2408P.$$

D , Down; R , Royal; T , Tallarook; F , Fir.

(iii) *Medium-weight Carcasses (35-36 lb).*—No significant improvement is obtained by using more than the two measurements F and Th for discriminating between grades at this weight (Fig. 4). The progressive increase in length of leg and depth of thorax accounts for all of

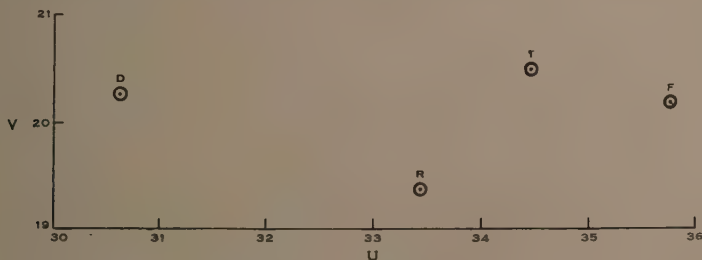


Fig. 4.—Maximum discrimination between grades of medium-weight carcasses (35-36 lb) obtained using functions of measurements F and Th .

$$U = 0.0719F + 0.0572Th,$$

$$V = -0.0562F + 0.1290Th.$$

D , Down; R , Royal; T , Tallarook; F , Fir.

the discrimination utilized between grades. The value for V is relatively uniform for all grades other than Royal. In commercial grading, discrimination between Down and Royal grades of carcasses of over 35 lb weight is made almost entirely on the basis of blockiness, of which length of leg is a good index. Grading between Royal, Tallarook, and Fir grades

depends primarily on fat cover. There is a natural tendency for thinner fat cover to go with longer legs, so that although length of leg does tend to increase with lowered grade, the tendency of the grader is to include in the Royal grade carcasses long in the leg, if they are well covered with fat. Table 4 shows the range of leg length in Royal and Tallarook grades to be the same, although the mean for the former is 6 mm less. The relatively long mean leg-length of Royal grade accounts for much of its anomalous situation in the *V*-dimension.

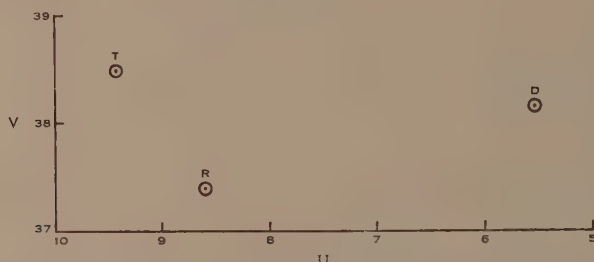


Fig. 5.—Maximum discrimination between grades of heavy-weight carcasses (43-44 lb) obtained using functions of measurements *F*, *K*, *W*, and *P*.

$$U = 0.0633F + 0.0022K - 0.0307W - 0.2339P,$$

$$V = -0.0412F + 0.0463K + 0.0683W - 0.2184P.$$

D, Down; R, Royal; T, Tallarook.

(iv) *Heavy-weight Carcasses* (43-44 lb).—Significant increases in discriminating power are obtained by successive inclusion of each of the four measurements *F*, *K*, *W*, and *P* (Fig. 5). The appearance of *W* in the useful measurements is due to the appreciably greater width of shoulder of Down grade as compared with lower grades. This is not necessarily desirable from the point of view of carcass quality. It appears that at these weights, excessive fat deposition may occur on the shoulder of Down type carcasses. To the eye such carcasses appear attractive and evenly balanced, but they would probably be excessively heavy in the forequarters, and hence wasteful.

In the *V*-dimension, Royal grade is again displaced for the same reasons as for the medium-weight carcasses.

IV. DISCUSSION

The value of measurements for the objective appraisal of a carcass depends on: their ease of taking; their repeatability; their relation to some aspect of carcass "quality"; and a correct assessment of the relative value of each measurement used.

Each of the four measurements found to be most valuable for overall discrimination was fairly easily made. Even the probe measure for thickness of loin (*BC*) was relatively simple, and repeatable results were readily

TABLE 9

COMPARISON OF MEASUREMENTS MADE ON CUT AND UNCUT CARCASSES

F, *G*, *Th*, comparable external measurements made by different workers. *BC*, *P*, probe measurements reported in this paper compared with the sums of *B* + *C* and *X* + *Y* respectively, as made on cut carcasses by other authors

Characteristic	Author*	Type of Carcass			
		Down		Royal	
Breed	1	Commercial carcasses of unknown breeding		Commercial carcasses of unknown breeding	
	2	Southdown × (Border Leicester-Cheviot)		Border Leicester × Blackfaced	
	3	Southdown × Corriedale		Romney Marsh × Corriedale	
	4	Commercial carcasses of unknown breeding		Commercial carcasses of unknown breeding	
	5	Southdown × (Romney or Corriedale)		(Romney, Border Leicester, Southdown, Suffolk, or Dorset Horn) × Corriedale	
		Medium Weight	Heavy Weight	Medium Weight	Heavy Weight
Weight of carcass (lb)	1	35-36	43-44	35-36	43-44
	2	38	44	38	43
	3	32-36	36-42	32-36	36-42
	4	36	43-50	36	43-50
	5	35-37	—	35-37	39-42
Length of leg (mm) (<i>F</i>)	1	223	232	257	262
	2	238	244	255	258
	3	230	230	245	256
	4	—	—	—	—
	5	240	—	256	267
Width of gigots (mm) (<i>G</i>)	1	211	219	211	221
	2	224	237	219	227
	3	220	223	217	223
	4	211	229	212	234
	5	224	—	218	234
Depth of thorax (mm) (<i>Th</i>)	1	255	270	262	280
	2	250	260	257	264
	3	248	252	253	264
	4	—	—	—	—
	5	—	—	—	—
Thickness of loin (mm) (<i>BC</i> = <i>B</i> + <i>C</i>)	1	33	37	32	34
	2	33.7	37.2	32.3	35.5
	3	—	—	—	—
	4	32.1	37.9	29.3	32.9
	5	—	—	—	—
Thickness of flank (mm) (<i>P</i> = <i>X</i> + <i>Y</i>)	1	16	15	20	16
	2	16.0	15.8	17.7	16.3
	3	—	—	—	—
	4	—	—	—	—
	5	—	—	—	—

*1, Robinson, Binet, and Doig (this paper). 2, Pálsson (1940). 3, Walker and McMeekan (1944). 4, Clarke and McMeekan (1952). 5, Coop and Clark (1952).

obtained. Only one of us (T.J.R.) made the measurements, so that no assessment of between-operator errors is possible.

Pålsson (1939, 1940), Walker and McMeekan (1944), and Clarke and McMeekan (1952) agree that measurements made at the last rib on the cut face of the carcass are extremely valuable, and the probe measures BC and P used on our intact carcasses were intended to take the place of $B + C$ (thickness of eye muscle + fat cover) and $X + Y$ (thickness of flesh + fat in the loin). That the probe measurements were indeed a fair estimate of $B + C$ and $X + Y$ is indicated by comparison of our results with those obtained from cut carcasses of comparable weight and quality by these workers (Table 9). This table is interesting also in that it clearly indicates that the top grade Victorian fat lamb compares very favourably with its New Zealand and English counterpart as regards shortness of leg and thickness of fleshing.

TABLE 10
INDICES OF "BLOCKINESS" OBTAINED USING THE RATIO OF LENGTH OF LEG (F) TO WIDTH OF GIGOTS (G) OR TWIST ($10\sqrt{Z}$)

Index	Grade:	Down			Royal			
	Wt. (lb):	27-28	35-36	43-44	27-28	35-36	43-44	51-52
G/F		0.94	0.94	0.95	0.88	0.82	0.84	0.90
$10\sqrt{Z}/F$		0.72	0.70	0.70	0.67	0.61	0.61	0.66

Index	Grade:	Tallarook			Fir	
	Wt. (lb):	27-28	35-36	43-44	27-28	35-36
G/F		0.79	0.81	0.83	0.77	0.77
$10\sqrt{Z}/F$		0.60	0.60	0.60	0.57	0.56

Pålsson (1939, 1940), Walker and McMeekan (1944), and Coop and Clark (1952) have used the ratio of width of gigots to length of leg (G/F) as an index of blockiness. F is a reflection of two things, namely, length of bone and the development of muscle within the crutch. Consequently it is markedly affected by the interaction between breed and environment, and could be expected to be of great value for purposes of discrimination. G is primarily a measure of the width of the pelvis and to a lesser extent of the muscle and fat cover over it, and from our data appears to have no value for discrimination between grades of carcasses. It is basically related to weight. Its usefulness, as used by the authors cited above, lies in the fact that its value relative to weight ($G/\text{wt.}$) *decreases* about 15 per cent. with each 8 lb increment in weight, regardless of grade. The ratio $F/\text{wt.}$ varies in two ways. Firstly, within grades it *decreases* by an average of 16 per cent. with each 8 lb weight increment; secondly, it *increases* from 6 to 9 per cent. with each successively lower grade. Since both G and F bear approximately the same relationship to

weight, the use of the expression G/F more or less cancels out the weight component and results in a series of declining values, similar for each grade and applicable to all weights of carcasses (Table 10).

Similar relationships are shown by the expression $10\sqrt{(Z)/F}$, but Z is a difficult measurement to make.

The basic argument on which the present investigation rests is that commercial grades of lamb carcasses do in fact represent grades of "quality". Presumably then, any measurements which are of value in differentiating between samples of carcasses representing grades should also be of value in differentiating between individual carcasses on a "quality" basis. The analysis of the data has been such as to place the various measurements in order of value and to put weightings on each to give maximum discrimination. This has resulted in the emergence of but four measurements of real value for overall discrimination, the final assessment of which must await their correlation with carcass analyses.

Strong presumptive evidence of their real value is provided by the model illustrated in Figure 2, in which most anomalies can be explained on the basis of the actual practice of grading as carried out at the meat works. It has been pointed out that four tendencies are apparent in the Y -dimension. Firstly, the light-weight carcasses form a series somewhat different from the two others. In the separate within-weights analysis, up to five measurements give successively better discrimination between grades of these carcasses, as compared with the two measurements necessary to discriminate between grades only 8 lb heavier. There is a ready explanation for this apparent anomaly. Grading is done on two major criteria: fat cover and conformation. Light carcasses tend to be deficient in fat, and a high proportion are rejected into local-market grades. Grading therefore becomes very subjective, and the grader, probably unconsciously, takes into account many things when deciding into which grade to put a light carcass. This is clearly reflected in the high degree of expected misclassification between grades (Table 8). This accounts also for part of the next two apparent anomalies, namely, the relatively good discrimination between Down and Royal grades of heavier carcasses, and the general skewness of the pattern in the Y -dimension. Heavier carcasses tend to be better covered with fat, and in an increasing proportion of cases fat cover ceases to be a factor in grading.

Full attention is then given to conformation in differentiating between Down and Royal grades. This has two effects. Firstly, it tends to give good discrimination on the basis of measurable conformation between the two top grades, and secondly, it tends to up-grade heavier carcasses. Fat carcasses of relatively poor conformation are included in export grades because their extra fat cover makes them appear more attractive. This natural tendency of the grader to favour heavier carcasses probably accounts for some of the skewness apparent in Figure 2.

The final anomaly, namely, the out-of-line placement of the very heavy Royals, is explained by the absence of a Down grade at these heavy weights (i.e. 51-52 lb). Down type carcasses are included in the Royal grade and so raise the grade average to an intermediate position.

In the *S*-dimension, the relative displacement of the medium- and heavy-weight Royals is caused by their relatively long legs as discussed above. This also contributes to the curvatures observed for these weights in the *XY*-plane.

TABLE 11
OBJECTIVE ASSESSMENT OF THE CONFORMATION OF THE FAT LAMB CARCASS BASED ON
VALUES OF Y'

$$Y' = 0.057F + 0.050Th - 0.097BC - 0.17P$$

$$\text{Score} = 250 - 10Y'$$

Means of 20 carcasses in each weight-grade classification

Grade	Wt. Category:	Light		Medium		Heavy		Very Heavy	
		Y'	Score	Y'	Score	Y'	Score	Y'	Score
Down		18.7	63	19.5	55	19.7	53	—	—
Royal		20.2	48	22.0	30	22.9	21	21.0	40
Tallarook		22.6	24	23.8	12	23.9	11	—	—
Fir		24.1	9	25.2	-2	—	—	—	—

The pattern which has emerged from the analysis is sufficiently good to warrant the tentative use of the formula for Y as it appears in the overall discrimination (Fig. 2), for the quantitative assessment of carcass conformation. In its full expression it is too complicated for practical use and in view of the nature of the material in use it appears to be justified to round off the formula to read

$$Y' = 0.057F + 0.050Th - 0.097BC - 0.17P.$$

The mean values for Y' , as they appear in Table 11, vary only from 18.7 to 25.2. In order to expand this difference it is convenient to express it as $250 - 10Y'$. One major objection to the use of this formula is the largely unexplained favouring of light-weight carcasses.

Final assessment of the value of these measurements, and of Y' in expressing their relative values, must await the results of carcass dissection and analysis. At least they form a picture which is understandable, and give a basis for future quantitative work.

V. ACKNOWLEDGMENTS

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